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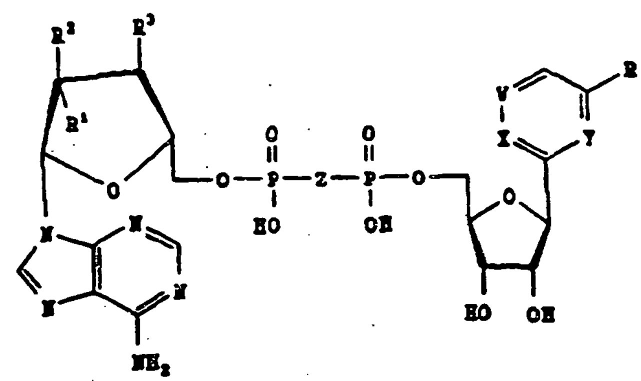
(54) Title: NICOTINAMIDE RIBONUCLEOTIDE ISOSTERS, ISOSTERIC NAD ANALOGUES. THEIR SYNTHESES AND USE IN TREATMENTS OF ALCOHOLISM AND NEOPLASTIC DISEASES

(57) Abstract

This invention provides a compound having structure (I) wherein R^1, R^2, and R^3 are same or different, and are hydrogen, hydroxyl, or fluorine; Z is O, CH_2 or CF_2; R is chlorine, bromine, iodine, carbonitrile, carboxylic ester, or carboxamide; and one of W, X and Y is N or N^+R', wherein R' is methyl or ethyl, and all others are CH; provided that R^1 is not H when R^2 and R^3 are OH, Z is O, R is carboxamide and W is N or N^+R'. This invention also provides methods of preparing the compounds and a method of treating a mammal having a NAD-dependent enzyme associated disorder.



(I)

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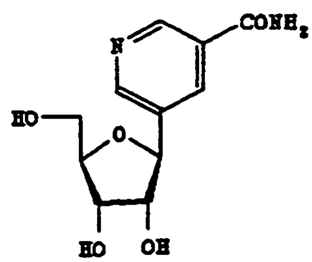
NICOTINAMIDE RIBONUCLEOTIDE ISOSTERS, ISOSTERIC NAD ANALOGUES. THEIR SYNTHESES AND USE IN TREATMENTS OF ALCOHOLISM AND NEOPLASTIC DISEASES.

The invention described herein was made in the course of work under Grant No. CA 33907 from the National Cancer Institute, National Institutes of Health, U. S. Department of Health and Human Services. The U.S. Government has certain rights in this invention.

BACKGROUND OF THE INVENTION

The pyridine C-nucleoside having the structure:



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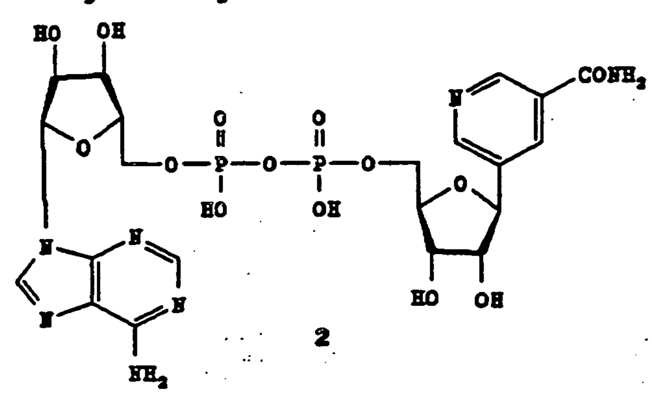
which is isosteric to nicotinamide riboside, was synthesized by these inventors [Kabat, Pankiewicz, Watanabe, *J. Med. Chem.*, 1987, *30*, 924-927; Kabat *et al.*, *Chem. Pharm. Bull.*, 1988, *36*, 634-640; Pankiewicz *et al.*, *J. Org. Chem.*, 1988, *53*, 3473-3479] in the hope that such an analogue may be converted biologically into the corresponding nicotinamide adenine dinucleotide, NAD coenzyme, analog having the structure:



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and exert biological activities. The non-charged NAD isostere (2), which is incapable of participating in biological oxidation-reduction process(es) may inhibit the NAD-dependent enzyme, IMP-dehydrogenase, and may induce anticancer activity by blocking the *de novo* GMP synthesis.

The NAD analog (2) which contains the C-nucleoside (1), was found to be a general competitive inhibitor (with respect to NAD) of various dehydrogenases such as inosine monophosphate dehydrogenase (IMPDH), glutamate dehydrogenase (GDH), lactate dehydrogenase (LDH) and malate dehydrogenase (MDH). Interestingly, the NAD analogue (2) exhibited highly potent and selective inhibitory activity against alcohol dehydrogenase from horse liver.

The present invention relates to the novel class of NAD analogs which contain the nicotinamide, picolinamide or isonicotinamide C-nucleoside in place of nicotinamide riboside. The compounds of this invention have the pyrophosphate (-P-O-P-) bridge connecting the nucleosides or, alternatively, can have a methylene diphosphonate ($-P-CH_2-P-$), or difluoromethylene diphosphonate ($-P-CF_2-P-$) group as the bridge.

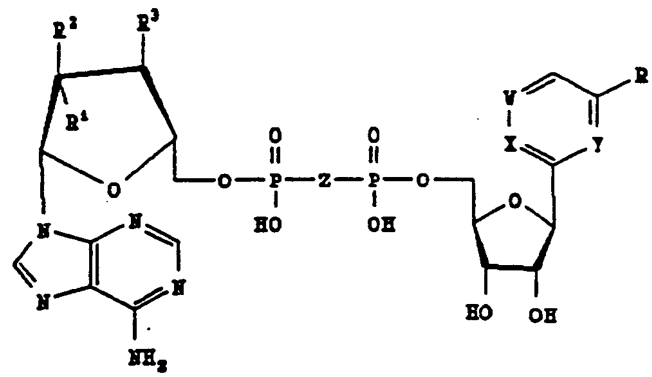
Analogues that contain a methylene diphosphonate ($-P-CH_2-P-$) or difluoromethylene diphosphonate ($-P-CF_2-P-$) group in place of the pyrophosphate (-P-O-P-) bridge are resistant to enzymic hydrolysis to their corresponding nucleoside 5′-monophosphates. The 2′-fluoroinated adenosine analogues cannot be converted into the corresponding NADP analogues. Such analogues, therefore, cannot interfere with NADP dependent enzymes.
In addition, fluorine substituted NAD analogues, as more lipophilic than their corresponding hydroxyl or pyrophosphate groups containing counterparts, could

penetrate biological membranes and may better fit to the hydrophobic binding pocket of dehydrogenases.

The compositions of this invention are useful as potent inhibitors of various dehydrogenases of eucaryotic and procaryotic origin. These compounds may also be utilized as therapeutic agents exhibiting anticancer and antiviral activity.

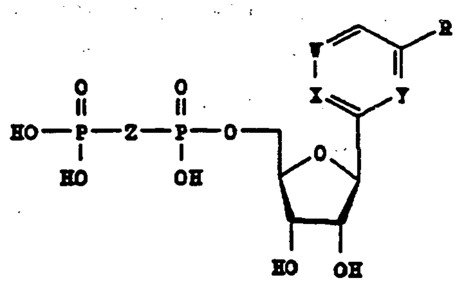
SUMMARY OF THE INVENTION

This invention provides a compound having the structure:



wherein R^1, R^2, and R^3 are same or different, and are hydrogen, hydroxyl, or fluorine; Z is O, CH_2 or CF_2; R is chlorine, bromine, iodine, carbonitrile, carboxylic ester, or carboxamide; and one of W, X and Y is N or N^+R', wherein R' is methyl or ethyl, and all others are CH; provided that R^1 is not H when R^2 and R^3 are OH, Z is O, R is carboxamide and W is N or N^+R'.

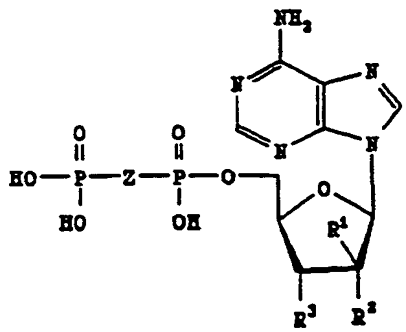
This invention also provides a compound having the structure:



wherein Z is CH_2 or CF_2; R is chlorine, bromine, iodine, carbonitrile, carboxylic ester, or carboxamide; and one of W, X and Y is N or

$N^{+}R'$, wherein R' is methyl or ethyl, and all others are CH.

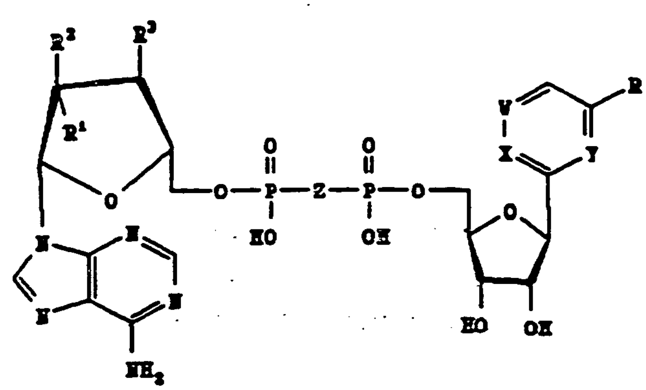
This invention also provides a compound having the structure:



wherein Z is CH_2 or CF_2; and R^1, R^2, and R^3 are same or different, and are hydrogen, hydroxyl, or fluorine.

This invention also provides a pharmaceutical composition which comprises any of the above-identified compounds and a pharmaceutically acceptable carrier.

This invention further provides a method of treating a mammal having a NAD-dependent enzyme associated disorder which comprises administering to the mammal a pharmaceutically effective amount of a compound having the structure:



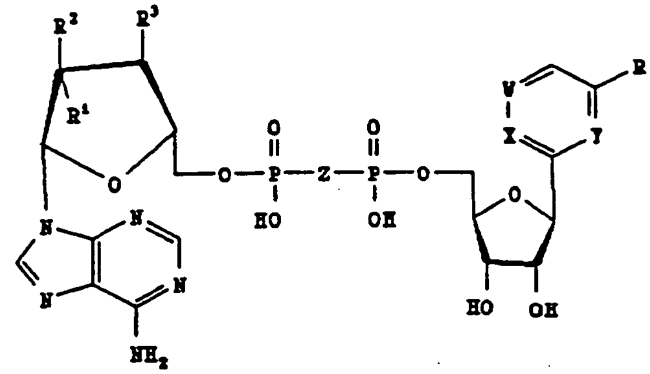
wherein R^1, R^2, and R^3 are same or different, and are hydrogen, hydroxyl, or fluorine; Z is O, CH_2 or CF_2; R is chlorine, bromine, iodine, carbonitrile, carboxylic ester, or carboxamide; and one of W, X and Y is N or N^+R', wherein R' is methyl or ethyl, and all others are CH;

effective to inhibit the NAD-dependent enzyme, thereby treating the disorder.

Finally, this invention provides methods of preparing the above-identified compounds.

DETAILED DESCRIPTION

This invention provides a compound having the structure:



wherein R^1, R^2, and R^3 are same or different, and are hydrogen, hydroxyl, or fluorine; Z is O, CH_2 or CF_2; R is chlorine, bromine, iodine, carbonitrile, carboxylic ester, or carboxamide; and one of W, X and Y is N or N^+R', wherein R' is methyl or ethyl, and all others are CH; provided that R^1 is not H when R^2 and R^3 are OH, Z is O, R is carboxamide and W is N or N^+R'.

Compounds having the above-identified structure, although not limited to the following compounds, may be selected from the group consisting of:

5-(ß-D-Ribofuranosyl)nicotinamide-(5'-5")-adenosine pyrophosphate,

6-(ß-D-Ribofuranosyl)picolinamide-(5'-5")-adenosine pyrophosphate,

2-(ß-D-Ribofuranosyl)isonicotinamide-(5'-5")-adenosine pyrophosphate,

5-(ß-D-Ribofuranosyl)-3-cyanopyridine-(5'-5")-adenosine pyrophosphate,

6-(β-D-Ribofuranosyl)-2-cyanopyridine-(5'-5")-adenosine pyrophosphate,

2-(β-D-Ribofuranosyl)-4-cyanopyridine-(5'-5")-adenosine pyrophosphate,

5-(β-D-Ribofuranosyl)nicotinamide-(5'-5")-2'-deoxyadenosine pyrophosphate,

6-(β-D-Ribofuranosyl)picolinamide-(5'-5")-2'-deoxyadenosine pyrophosphate,

2-(β-D-Ribofuranosyl)isonicotinamide-(5'-5")-2'-deoxyadenosine pyrophosphate,

5-(β-D-Ribofuranosyl)-3-cyanopyridine-(5'-5")-2'-deoxyadenosine pyrophosphate,

6-(β-D-Ribofuranosyl)-2-cyanopyridine-(5'-5")-2'-deoxyadenosine pyrophosphate,

2-(β-D-Ribofuranosyl)-4-cyanopyridine-(5'-5")-2'-deoxyadenosine pyrophosphate,

5-(β-D-Ribofuranosyl)nicotinamide-(5'-5")-2'-deoxy-2'-fluoroadenosine pyrophosphate,

6-(β-D-Ribofuranosyl)picolinamide-(5'-5")-2"-deoxy-2"-fluoroadenosine pyrophosphate,

2-(β-D-Ribofuranosyl)isonicotinamide-(5'-5")-2'-deoxy-2'-fluoroadenosine pyrophosphate,

5-(β-D-Ribofuranosyl)-3-cyanopyridine-(5'-5")-2'-deoxy-2'-fluoroadenosine pyrophosphate,

6-(β-D-Ribofuranosyl)-2-cyanopyridine-(5'-5")-2'-

deoxy-2'-fluoroadenosine pyrophosphate,

2-(β-D-Ribofuranosyl)-4-cyanopyridine-(5'-5")-2'-deoxy-2'-fluoroadenosine pyrophosphate,

5-(β-D-Ribofuranosyl)nicotinamide-(5'-5")-3'-deoxy-3'-fluoroadenosine pyrophosphate,

6-(β-D-Ribofuranosyl)picolinamide-(5'-5")-3'-deoxy-3'-fluoroadenosine pyrophosphate,

2-(β-D-Ribofuranosyl)isonicotinamide-(5'-5")-3'-deoxy-3'-fluoroadenosine pyrophosphate,

5-(β-D-Ribofuranosyl)-3-cyanopyridine-(5'-5")-3'-deoxy-3'-fluoroadenosine pyrophosphate,

6-(β-D-Ribofuranosyl)-2-cyanopyridine-(5'-5")-3'-deoxy-3'-fluoroadenosine pyrophosphate,

2-(β-D-Ribofuranosyl)-4-cyanopyridine-(5'-5")-3'-deoxy-3'-fluoroadenosine pyrophosphate,

5-(β-D-Ribofuranosyl)nicotinamide-(5'-5")-9-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)-adenine pyrophosphate,

6-(β-D-Ribofuranosyl)picolinamide-(5'-5")-9-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)-adenine pyrophosphate,

2-(β-D-Ribofuranosyl)isonicotinamide-(5'-5")-9-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)-adenine pyrophosphate,

5-(β-D-Ribofuranosyl)-3-cyanopyridine-(5'-5")-9-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)-adenine

pyrophosphate,

6-(β-D-Ribofuranosyl)-2-cyanopyridine-(5'-5")-9-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)-adenine pyrophosphate,

2-(β-D-Ribofuranosyl)-4-cyanopyridine-(5'-5")-9-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)-adenine pyrophosphate,

P^1-[5-(β-D-Ribofuranosyl)nicotinamide-5'-yl]-P^2-[adenosine-5"-yl]methylenediphosphonate,

P^1-[6-(β-D-Ribofuranosyl)picolinamide-5'-yl]-P^2-[adenosine-5"-yl]methylenediphosphonate,

P^1-[2-(β-D-Ribofuranosyl)isonicotinamide-5'-yl]-P^2-[adenosine-5"-yl]methylenediphosphonate,

P^1-[5-(β-D-Ribofuranosyl)-3-cyanopyridine-5'-yl]-P^2-[adenosine-5"-yl]methylenediphosphonate,

P^1-[6-(β-D-Ribofuranosyl)-2-cyanopyridine-5'-yl]-P^2-[adenosine-5"-yl]methylenediphosphonate,

P^1-[2-(β-D-Ribofuranosyl)-4-cyanopyridine-5'-yl]-P^2-[adenosine-5"-yl]methylenediphosphonate,

P^1-[5-(β-D-Ribofuranosyl)nicotinamide-5'-yl]-P^2-[2'-deoxyadenosine-5"-yl]methylenediphosphonate,

P^1-[6-(β-D-Ribofuranosyl)picolinamide-5'-yl]-P^2-[2'-deoxyadenosine-5"-yl]methylenediphosphonate,

P^1-[2-(β-D-Ribofuranosyl)isonicotinamide-5'-yl]-P^2-[2'-deoxyadenosine-5"-yl]methylenediphosphonate,

P^1-[5-(ß-D-Ribofuranosyl)-3-cyanopyridine-5'-yl]P^2-[2'-deoxyadenosine-5"-yl]methylenediphosphonate,

P^1-[6-(ß-D-Ribofuranosyl)-2-cyanopyridine-5'-yl]-P^2-2'-deoxyadenosine-5"-yl]methylenediphosphonate,

P^1-[2-(ß-D-Ribofuranosyl)-4-cyanopyridine-5'-yl]-P^2-[2'-deoxyadenosine-5"-yl]methylenediphosphonate,

P^1-[5-(ß-D-Ribofuranosyl)nicotinamide-5'-yl]-P^2-[2'-deoxy-2'-fluoroadenosine-5"-yl]-methylenediphosphonate,

P^1-[6-(ß-D-Ribofuranosyl)picolinamide-5'-yl]-P^2-[2'-deoxy-2'-fluoroadenosine-5"-yl]-methylenediphosphonate,

P^1-[2-(ß-D-Ribofuranosyl)isonicotinamide-5'-yl]-P^2-[2'-deoxy-2'-fluoroadenosine-5"-yl]-methylenediphosphonate,

P^1-[5-(ß-D-Ribofuranosyl)-3-cyanopyridine-5'-yl]-P^2-[2'-deoxy-2'-fluoroadenosine-5"-yl]-methylenediphosphonate,

P^1-[6-(ß-D-Ribofuranosyl)-2-cyanopyridine-5'-yl]-P^2-[2'-deoxy-2'-fluoroadenosine-5"-yl]-methylenediphosphonate,

P^1-[2-(ß-D-Ribofuranosyl)-4-cyanopyridine-5'-yl]-P^2-[2'-deoxy-2'-fluoroadenosine-5"-yl]-methylenediphosphonate,

P^1-[5-(ß-D-Ribofuranosyl)nicotinamide-5'-yl]-P^2-[3'-deoxy-3'-fluoroadenosine-5"-yl]-methylenediphosphonate,

P[1]-[6-(ß-D-Ribofuranosyl)picolinamide-5'-yl]-P[2]-[3'-deoxy-3'-fluoroadenosine-5"-yl]-methylenediphosphonate,

P[1]-[2-(ß-D-Ribofuranosyl)isonicotinamide-5'-yl]-P[2]-[3'-deoxy-3'-fluoroadenosine-5"-yl]-methylenediphosphonate,

P[1]-[5-(ß-D-Ribofuranosyl)-3-cyanopyridine-5'-yl]-P[2]-[3'-deoxy-3'-fluoroadenosine-5"-yl]-methylenediphosphonate,

P[1]-[6-(ß-D-Ribofuranosyl)-2-cyanopyridine-5'-yl]-P[2]-[3'-deoxy-3'-fluoroadenosine-5"-yl]-methylenediphosphonate,

P[1]-[2-(ß-D-Ribofuranosyl)-4-cyanopyridine-5'-yl]-P[2]-[3'-deoxy-3'-fluoroadenosine-5"-yl]-methylenediphosphonate,

P[1]-[5-(ß-D-Ribofuranosyl)nicotinamide-5'-yl]-P[2]-[9-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)adenine-5"-yl]methylenediphosphonate,

P[1]-[6-(ß-D-Ribofuranosyl)picolinamide-5'-yl]-P[2]-[9-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)adenine-5"-yl]methylenediphosphonate,

P[1]-[2-(ß-D-Ribofuranosyl)isonicotinamide-5'-yl]-P[2]-[9-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)adenine-5"-yl]methylenediphosphonate,

P[1]-[5-(ß-D-Ribofuranosyl)-3-cyanopyridine-5'-yl]-P[2]-[9-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)adenine-5"-yl]methylenediphosphonate,

P[1]-[6-(ß-D-Ribofuranosyl)-2-cyanopyridine-5'-yl]-P[2]-

[9-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)adenine-5″-yl]methylenediphosphonate,

P¹-[2-(β-D-Ribofuranosyl)-4-cyanopyridine-5′-yl]-P²-[9-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-adenine-5″-yl]methylenediphosphonate,

P¹-[5-(β-D-Ribofuranosyl)nicotinamide-5′-yl]-P²-[adenosine-5″-yl]difluoromethylenediphosphonate,

P¹-[6-(β-D-Ribofuranosyl)picolinamide-5′-yl]-P²-[adenosine-5″-yl]difluoromethylenediphosphonate,

P¹-[2-(β-D-Ribofuranosyl)isonicotinamide-5′-yl]-P²-[adenosine-5″-yl]difluoromethylenediphosphonate,

P¹-[5-(β-D-Ribofuranosyl)-3-cyanopyridine-5′-yl]-P²-[adenosine-5″-yl]difluoromethylenediphosphonate,

P¹-[6-(β-D-Ribofuranosyl)-2-cyanopyridine-5′-yl]-P²-[adenosine-5″-yl]difluoromethylenediphosphonate,

P¹-[2-(β-D-Ribofuranosyl)-4-cyanopyridine-5′-yl]-P²-[adenosine-5″-yl]difluoromethylenediphosphonate,

P¹-[5-(β-D-Ribofuranosyl)nicotinamide-5′-yl]-P²-[2′-deoxyadenosine-5″-yl]difluoromethylenediphosphonate,

P¹-[6-(β-D-Ribofuranosyl)picolinamide-5′-yl]-P²-[2′-deoxyadenosine-5″-yl]difluoromethylenediphosphonate,

P¹-[2-(β-D-Ribofuranosyl)isonicotinamide-5′-yl]-P²-[2′-deoxyadenosine-5″-yl]-difluoromethylenediphosphonate,

P^1-[5-(ß-D-Ribofuranosyl)-3-cyanopyridine-5'-yl]P^2-[2'-deoxyadenosine-5"-yl]difluoromethylenediphosphonate,

P^1-[6-(ß-D-Ribofuranosyl)-2-cyanopyridine-5'-yl]-P^2-2'-deoxyadenosine-5"-yl]difluoromethylenediphosphonate,

P^1-[2-(ß-D-Ribofuranosyl)-4-cyanopyridine-5'-yl]-P^2-[2'-deoxyadenosine-5"-yl]difluoromethylenediphosphonate,

P^1-[5-(ß-D-Ribofuranosyl)nicotinamide-5'-yl]-P^2-[2'-deoxy-2'-fluoroadenosine-5"-yl]difluoromethylenediphosphonate,

P^1-[6-(ß-D-Ribofuranosyl)picolinamide-5'-yl]-P^2-[2'-deoxy-2'-fluoroadenosine-5"-yl]difluoromethylenediphosphonate,

P^1-[2-(ß-D-Ribofuranosyl)isonicotinamide-5'-yl]-P^2-[2'-deoxy-2'-fluoroadenosine-5"-yl]-difluoromethylenediphosphonate,

P^1-[5-(ß-D-Ribofuranosyl)-3-cyanopyridine-5'-yl]-P^2-[2'-deoxy-2'-fluoroadenosine-5"-yl]-difluoromethylenediphosphonate,

P^1-[6-(ß-D-Ribofuranosyl)-2-cyanopyridine-5'-yl]-P^2-[2'-deoxy-2'-fluoroadenosine-5"-yl]-difluoromethylenediphosphonate,

P^1-[2-(ß-D-Ribofuranosyl)-4-cyanopyridine-5'-yl]-P^2-[2'-deoxy-2'-fluoroadenosine-5"-yl]-difluoromethylenediphosphonate,

P^1-[5-(ß-D-Ribofuranosyl)nicotinamide-5'-yl]-P^2-[3'-

deoxy-3'-fluoroadenosine-5"-yl]-difluoromethylenediphosphonate,

P^1-[6-(β-D-Ribofuranosyl)picolinamide-5'-yl]-P^2-[3'-deoxy-3'-fluoroadenosine-5"-yl]-difluoromethylenediphosphonate,

P^1-(2-β-D-Ribofuranosyl)isonicotinamide-5'-yl-P^2-3'-deoxy-3'-fluoroadenosine-5"-yl]-difluoromethylenediphosphonate,

P^1-[5-(β-D-Ribofuranosyl)-3-cyanopyridine-5'-yl]-P^2-[3'-deoxy-3'-fluoroadenosine-5"-yl]-difluoromethylenediphosphonate,

P^1-[6-(β-D-Ribofuranosyl)-2-cyanopyridine-5'-yl]-P^2-[3'-deoxy-3'-fluoroadenosine-5"-yl]-difluoromethylenediphosphonate,

P^1-[2-(β-D-Ribofuranosyl)-4-cyanopyridine-5'-yl]-P^2-[3'-deoxy-3'-fluoroadenosine-5"-yl]-difluoromethylenediphosphonate,

P^1-[5-(β-D-Ribofuranosyl)nicotinamide-5'-yl]-P^2-[9-(2'-deoxy-2'-fluoro-β-D-arabino-furanosyl)]adenine-5"-yl]-difluoromethylenediphosphonate,

P^1-[6-(β-D-Ribofuranosyl)picolinamide-5'-yl]-P^2-[9-(2'-deoxy-2'-fluoro-β-D-arabino-furanosyl)]adenine-5"-yl]-difluoromethylenediphosphonate,

P^1-[2-(β-D-Ribofuranosyl)isonicotinamide-5'-yl]-P^2-[9-(2'-deoxy-2'-fluoro-β-D-arabino-furanosyl)-adenine-5"-yl]-difluoromethylenediphosphonate,

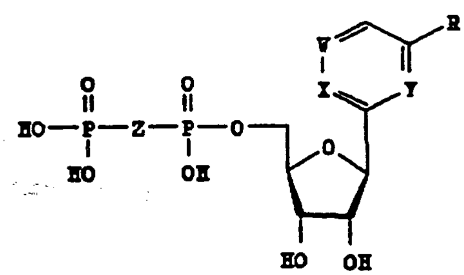
P^1-[5-(β-D-Ribofuranosyl)-3-cyanopyridine-5'-yl]-P^2-[9-(2'-deoxy-2'-fluoro-β-D-arabino-furanosyl)-

adenine-5"-yl]-difluoromethylenediphosphonate,

P^1-[6-(ß-D-Ribofuranosyl)-2-cyanopyridine-5'-yl]-P^2-[9-(2'-deoxy-2'-fluoro-β-D-arabino-furanosyl)-adenine-5"-yl]-difluoromethylenediphosphonate, and

P^1-[2-(ß-D-Ribofuranosyl)-4-cyanopyridine-5'-yl]-P^2-[9-(2'-deoxy-2'-fluoro-β-D-arabino-furanosyl)-adenine-5"-yl]-difluoromethylenediphosphonate.

This invention also provides a compound having the structure:



wherein Z is CH_2 or CF_2; R is chlorine, bromine, iodine, carbonitrile, carboxylic ester, or carboxamide; and one of W, X and Y is N or N^+R', wherein R' is methyl or ethyl, and all others are CH.

Compounds having the above-identified structure, although not limited to the following compounds, may be selected from the group consisting of:

(5-ß-D-Ribofuranosylnicotiamide-5'-yl)methylenediphosphonate

(6-ß-D-Ribofuranosylpicolinamide-5'-yl)methylenediphosphonate,

(2-ß-D-Ribofuranosylisonicotinamide-5'-yl)methylene-diphosphonate,

(5-ß-D-Ribofuranosyl-3-cyanopyridine-5'-yl)methylene-diphosphonate,

(6-ß-D-Ribofuranosyl-2-cyanopyridine-5'-yl)methylene-diphosphonate,

(2-(ß-D-Ribofuranosyl-4-cyanopyridine-5'-yl)methylene-diphosphonate,

(5-ß-D-Ribofuranosylnicotinamide-5'-yl)difluoromethylene-diphosphonate,

(6-ß-D-Ribofuranosylpicolinamide-5'-yl)difluoromethylene-diphosphonate,

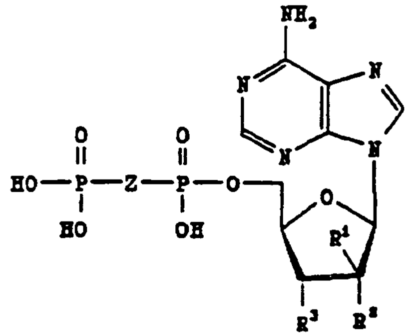
(2-ß-D-Ribofuranosylisonicotinamide-5'-yl)difluoromethylene-diphosphonate,

(5-ß-D-Ribofuranosyl-3-cyanopyridine-5'-yl)difluoromethylene-diphosphonate,

(6-ß-D-Ribofuranosyl-2-cyanopyridine-5'-yl)difluoromethylene-diphosphonate, and

(2-(ß-D-Ribofuranosyl-4-cyanopyridine-5'-yl)difluoromethylene-diphosphonate.

This invention also provides a compound having the structure:



wherein Z is CH_2 or CF_2; and R^1, R^2, and R^3 are same or different, and are hydrogen, hydroxyl, or fluorine.

Compounds having the above-identified structure, although not limited to the following compounds, may be selected from the group consisting of:

(Adenosin-5'-yl)methylenediphosphonate,

(2'-deoxyadenosin-5'-yl)methylenediphosphonate,

(2'-deoxy-2'-fluoroadenosin-5'-yl)methylenediphosphonate,

(3'-deoxy-3'-fluoroadenosin-5'-yl)methylenediphosphonate,

[9-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)adenine-5'-yl]methylenediphosphonate,

(Adenosin-5'-yl)difluoromethylenediphosphonate,

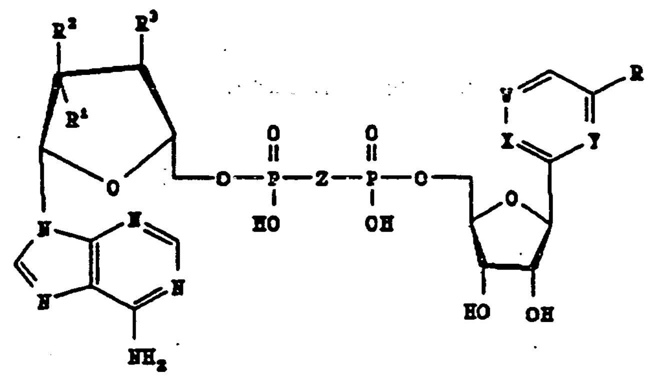
(2'-deoxyadenosin-5'-yl)difluoromethylenediphosphonate,

(2'-deoxy-2'-fluoroadenosin-5'-yl)difluoromethylene-diphosphonate,

(3'-deoxy-3'-fluoroadenosin-5'-yl)difluoromethylene-diphosphonate, and

[9-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)adenine-5'-yl]difluoromethylenediphosphonate.

This invention further provides a method of treating a mammal having a NAD-dependent enzyme associated disorder which comprises administering to the mammal a pharmaceutically effective amount of a compound having the structure:



wherein R^1, R^2, and R^3 are same or different, and are hydrogen, hydroxyl, or fluorine; Z is O, CH_2 or CF_2; R is chlorine, bromine, iodine, carbonitrile, carboxylic ester, or carboxamide; and one of W, X and Y is N or N^+R', wherein R' is methyl or ethyl, and all others are CH;

effective to inhibit the NAD-dependent enzyme, thereby treating the disorder.

For the purpose of this invention, the term "NAD-

dependent enzyme" means an enzyme which requires the presence of the co-enzyme NAD in order to assist the enzymatic reaction. Examples of enzymes which are dependent on NAD are known to those skilled in the art and include, but are not limited to, malate dehydrogenase, lactate dehydrogenase, alcohol dehydrogenase, inosine monophosphat dehydrogenase, glutamate dehydrogenase, isocitrate dehydrogenase, 6-phosphogluconate dehydrogenase, aldehyde dehydrogenase, dihydrosteroid dehydrogenase and dihydrofolate reductase.

For the purposes of this invention, the term "NAD-dependent enzyme associated disorder" is any disorder which arises or is aggrivated due to the enzymatic action of an NAD-dependent enzyme. Examples of such disorders are readily determinable by those skilled in the art.

In a preferred embodiment of this invention the NAD-dependent enzyme is alcohol dehydrogenase. In a second preferred embodiment of this invention the NAD-dependent enzyme is inosine monophosphate dehydrogenase.

Examples of disorders associated with the enzymatic action of alcohol dehydrogenase include, but are not limited to, acute alcohol posioning from the ingestion of such substances as ethanol, methanol or isopropyl alcohol, ethylene glycol intoxication, ethanol-induced hypoglycemia and lactacidemia.

Examples of disorders associated with the enzymatic action of inosine monophosphate dehydrogenase include, but are not limited to disorders characterized by the proliferation of malignant cells. Examples of disorders which are associated with the proliferation of malignant cells to which the compounds of the subject invention would be effective are readily determinable by those skilled in the art and include, but are not limited to,

cancers of the breast, colon, stomach, pancreas, ovary, head and neck, and urinary bladder, leukemias such as acute lymphocytic, acute granulocytic and chronic granulocytic leukemias, hairy cell leukemia, chronic lymphocytic leukemia, and other malignant disorders such as mycosis fungoides.

This invention also provides a pharmaceutical composition which comprises any of the above-identified compounds and a pharmaceutically acceptable carrier. In the preferred embodiment of this invention, the compounds are administered to the mammal as a pharmaceutical composition.

As used herein, the term "pharmceutically acceptable carrier" encompasses any of the standard pharmaceutical carriers such as an organic or inorganic inert carrier material suitable for enteral or parenteral administration which include, but are not limited to, water, gelatin, gum arabic, lactose, starches, magnesium stearate, talc, vegetable oils, polyalkylene glycols, petroleum gelly, etc. The pharmacological preparations can be made up in solid form such as tablets, dragees, suppositories or capsules, or in liquid form such as solutions, suspensions, or emulsions. The preparations may be sterilized and/or contain adjuvants such as preserving, stabilizing, wetting or emulsifying agents, salts for varying the osmotic pressure, or buffers. Such preparations may also contain other therapeutic agents.

For the purposes of this invention, the term "pharmaceutically effecitve amount" of the compound means any amount of the compound which, when incorporated in the pharmaceutical composition, will be effective to inhibit the enzymatic action of an NAD-dependent enzyme and, thereby, treat an NAD-dependent enzyme associated disorder but less than an amount which would be toxic to

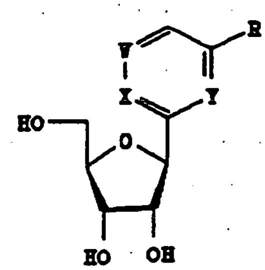
the mammal. In the practice of this invention the amount of the compound incorporated in the pharmaceutical composition may vary widely. Factors considered when determining the precise amount are well known to those skilled in the art. Examples of such factors include, but are not limited to, the subject being treated, the specific pharmaceutical carrier and route of administration being employed and the frequency with which the composition is to be administered. In a preferred embodiment of this invention, the pharmaceutically effective amount of the compound is in the range of 10 picomolar to 10 milimolar. In a particularly preferred embodiment the pharmaceutically effective amount is in the range of 10 micromolar.

In the practice of this invention, the administration of the composition may be effected by any of the well known methods including, but not limited to, oral, intravenous, intraperitoneal, intramuscular or subcutaneous or topical administration.

The compounds of this invention are prepared according to the following methods.

In the first method, the nucleosides are converted to their corresponding 5′-monophosphates and then coupled together to form the dinucleotides of this invention as follows:

(a) reacting a compound having the structure:

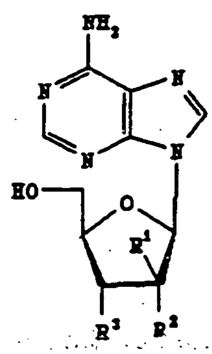


Formula II

wherein R is chlorine, bromine, iodine, carbonitrile, carboxylic ester, or carboxamide; and one of W, X and Y is N or $N^{+}R'$, wherein R' is methyl or ethyl, and all others are CH;

with phosphorous oxychloride in triethylphosphate under suitable conditions to form the nucleoside 5'-monophosphate derivative of the compound;

(b) reacting a compound having the structure:

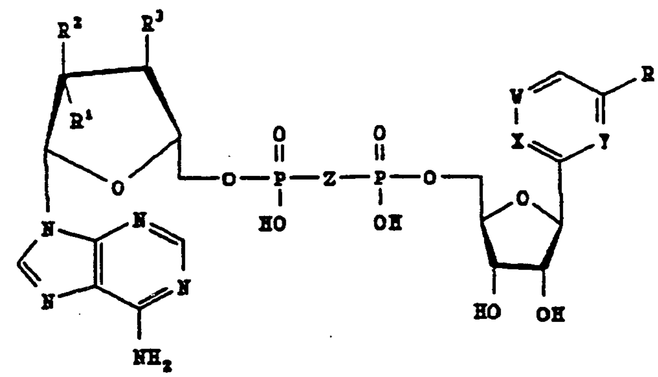


Formula III

wherein R^1, R^2, and R^3 are same or different, and are hydrogen, hydroxyl, or fluorine;

with phosphorous oxychloride in triethylphosphate under suitable conditions to form the nucleoside 5'-monophosphate derivative of the compound; and

(c) reacting the 5'-monophosphate derivative formed in step (a) or (b) with carbonyldiimidazole or dicyclohexyl carbodiimide under suitable conditions to activate the 5'-monophosphate derivative and then contacting the activated compound with an unactivated compound of step (b) or (a), respectively, to form a compound having the structure:

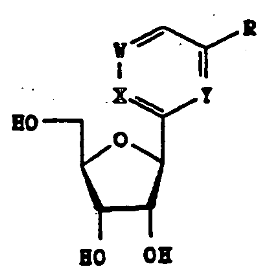


wherein R^1, R^2, and R^3 are same or different, and are hydrogen, hydroxyl, or fluorine; Z is O; R is chlorine, bromine, iodine, carbonitrile, carboxylic ester, or carboxamide; and one of W, X and Y is N or N^+R', wherein R' is methyl or ethyl, and all others are CH;

This method proceeds along the lines of known methods for the formation of the 5'-monophosphate derivatives of nucleosides; Yoshikawa et al., Tetrahedron Letters, 1967, **19**, 5065-5068.

The dinucleotides of this invention, wherein Z is CH_2 or CF_2, can also be prepared by first forming the 5'-methylene-diphosphonate or 5'-difluoromethylene-diphosphonate derivative nucleosides of this invention and then coupling the derivatives to the corresponding nucleosides bearing the 5'-hydroxy group. The method proceeds as follows:

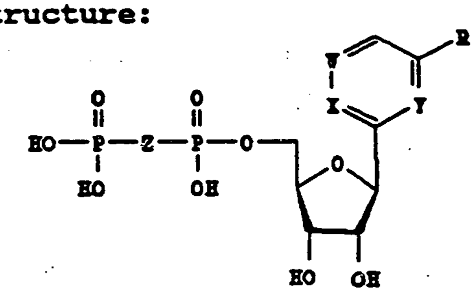
(a) reacting a compound having the structure:



wherein R is chlorine, bromine, iodine, carbonitrile, carboxylic ester, or carboxamide; and one of W, X and Y is N or $N^{+}R'$, wherein R' is methyl or ethyl, and all others are CH;

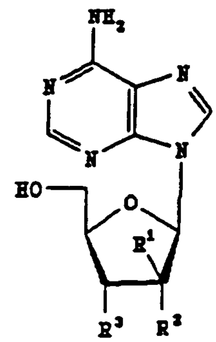
with the precursor of a suitable protecting group under suitable conditions to form the 2'3'-O-protected nucleoside; and

(b) reacting the compound formed in step (a) with methylenediphosphonate tetrachloride in triethylphosphate under suitable conditions to form the 5'-methylenediphosphonate derivative, which, after reacting under suitable conditions to selectively remove the 2',3'-O-protecting groups, has the structure:



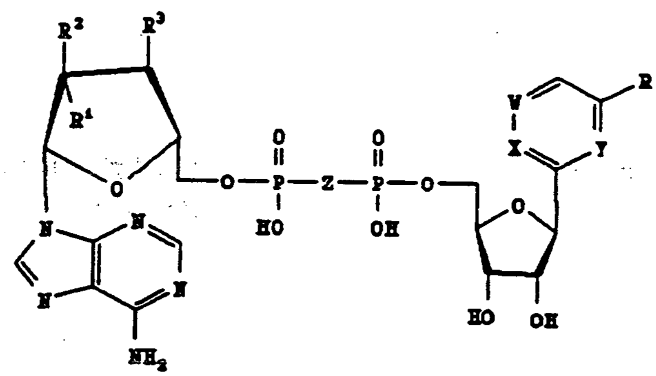
wherein Z is CH_2; R is chlorine, bromine, iodine, carbonitrile, carboxylic ester, or carboxamide; and one of W, X and Y is N or $N^{+}R'$, wherein R' is methyl or ethyl, and all others are CH.

The dinucleotides of the invention are then prepared by reacting the 5'-methylenediphosphonate derivative formed above with the nucleoside having the structure:



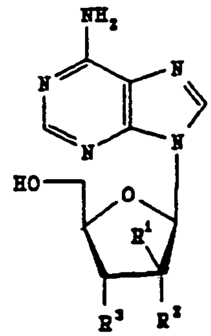
wherein R^1, R^2, and R^3 are same or different, and are hydrogen, hydroxyl, or fluorine;

under suitable conditions to allow for the coupling of the nucleosides to form the compound having the structure:



wherein Z is CH_2; R is chlorine, bromine, iodine, carbonitrile, carboxylic ester, or carboxamide; R^1, R^2, and R^3 are same or different, and are hydrogen, hydroxyl, or fluorine; and one of W, X and Y is N or N^+R', wherein R' is methyl or ethyl, and all others are CH.

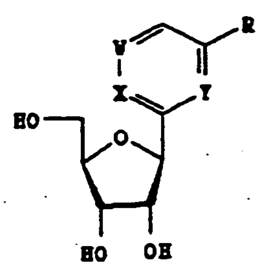
The 5'-methylenediphosphonate derivative of the compound having the structure:



wherein R^1, R^2, and R^3 are same or different, and are hydrogen, hydroxyl, or fluorine;

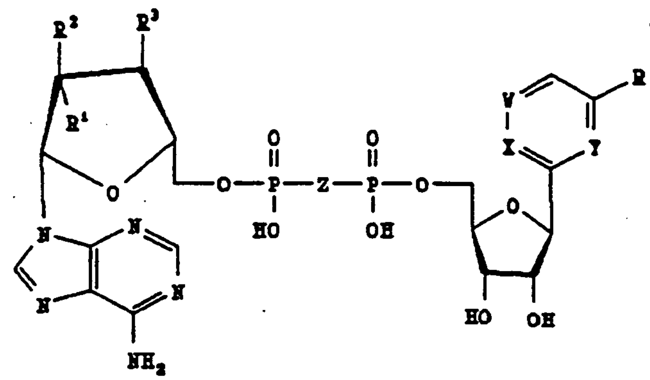
is formed by the same method as above, wherein step (a) and the deprotection in step (b) are necessary only if any of R^1, R^2, and R^3 are hydroxyl groups.

The dinucleotides of the invention are then prepared by reacting the 5'-methylenediphosphonate derivative formed above with the nucleoside having the structure:



wherein R is chlorine, bromine, iodine, carbonitrile, carboxylic ester, or carboxamide; and one of W, X and Y is N or N^+R', wherein R' is methyl or ethyl, and all others are CH;

under suitable conditions to allow for the coupling of the nucleosides to form the compound having the structure:



wherein Z is CH_2; R is chlorine, bromine, iodine, carbonitrile, carboxylic ester, or carboxamide; R^1, R^2, and R^3 are same or different, and are hydrogen, hydroxyl, or fluorine; and one of W, X and Y is N or N^+R', wherein R' is methyl or ethyl, and all others are CH.

In this method the reaction of step (a) comprises the selective protection of the vicinal cis hydroxy groups on the nucleoside to leave the 5'hydroxyl group as the remaining reactive site. For the purpose of this invention, a "precursor of a suitable protecting group" will comprise any compound that can be reacted with the compound of step (a) to allow for selective replacement of the vicinal hydroxyl cis hydroxy group with the corresponding O-protecting group. Examples of these are well known to those skilled in the art and include, but are not limited to, such compounds as isopropylidene and ethyl orthoformate. In this step the molar ratio of the reactants is in the range of 1:10 to 1:100 and the reaction is carried out at a temperature range of 0 °C to 50 °C for a period of 5 minutes to 2 days.

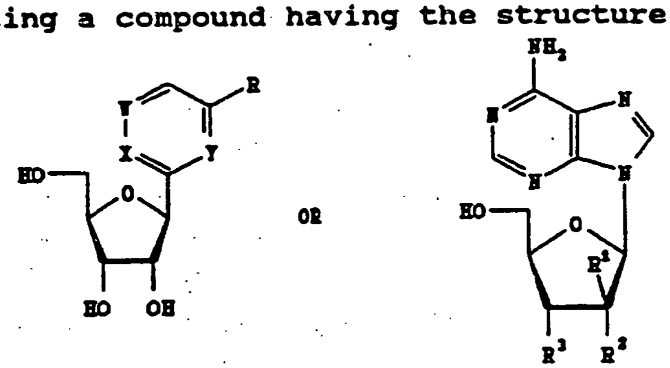
The reaction of step (b) comprises the formation of the

5′-methylenediphosphonate derivatives by reacting the compound formed in step (a) with methylenediphosphonate tetrachloride in triethylphosphate. In this step the molar ratio of the reactants is in the range of 2:1 to 1:10 and the reaction is carried out at a temperature range of -20 °C to 50 °C for a period of 5 minutes to 10 hours. Step (b) also comprises the selective removal of any protecting groups to form the 2′,3′-hydroxy group substituents. The conditions of this step comprise acidic hydrolysis using Dowex 50 (H^+), organic acid such as acetic acid, trifluoroacetic acid an the like, or inorganic acid such as hydrochloric acid, sulfuric acid and the like.

The coupling of the nucleosides is then carried out under reaction conditions of . . . .

This invention also provides another method of making the dinucleotides of this invention wherein Z is CH2 or CF2 which proceeds as follows:

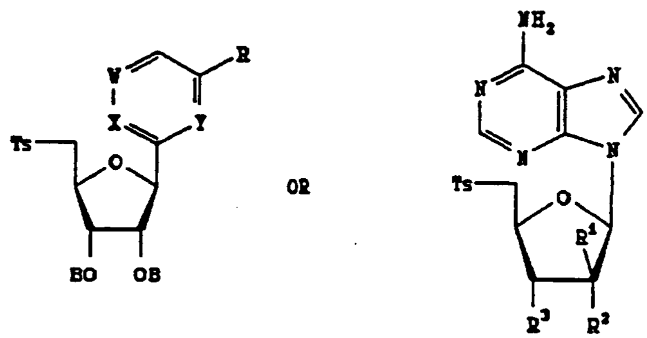
a) reacting a compound having the structure:



wherein R, R^1, R^2, R^3, W, X and Y are the same as defined previously;

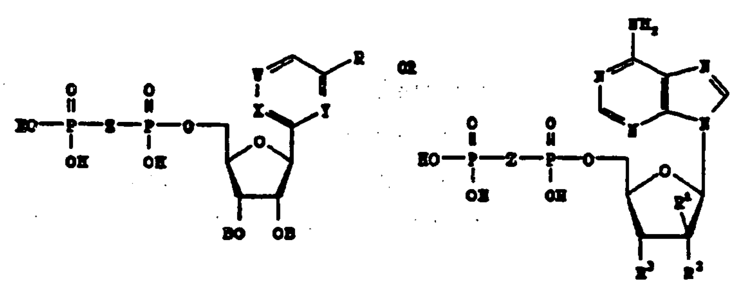
with the precursor of a suitable protecting group to under suitable conditions to selectively protect the 2′ and 3′ hydroxyl groups on the compounds;

b) reacting the compounds formed in step (a) with tosyl chloride under tosylating conditions to form the compounds having the structure:



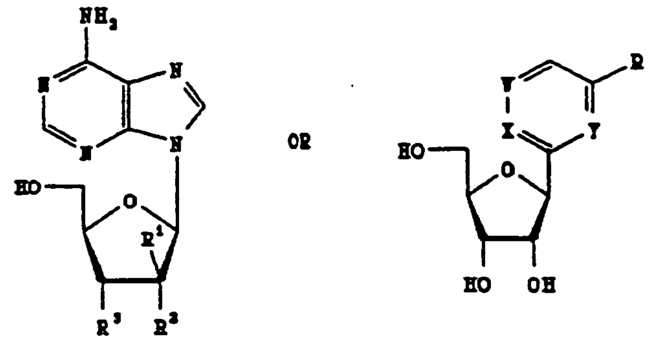
wherein Ts is tosyl and R, W, X and Y are the same as defined previously B is a protecting group and R^1, R^2, and R^3 are hydrogen, fluorine or an O-protecting group;

c) reacting the compound formed in step (b) with tris(tetra-n-butylammonium) methylene diphosphonate or tris(tetra-n-butylammonium) difluoromethylene diphosphonate in dimethylsulfoxide to form compounds having the structure:



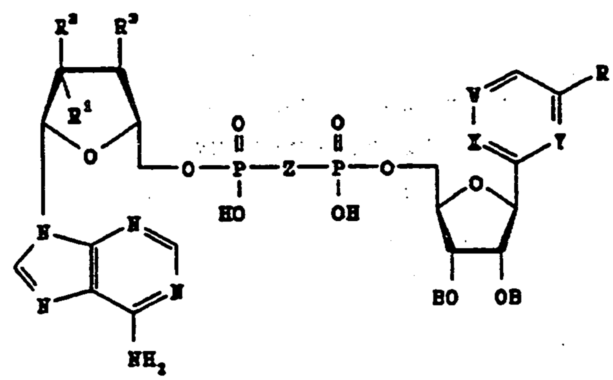
wherein Z is CH_2 or CF_2 and B, R, R^1, R^2, R^3, W, X and Y are the same as defined previously;

d) reacting the compound formed in step (c) with a compound having the structure:



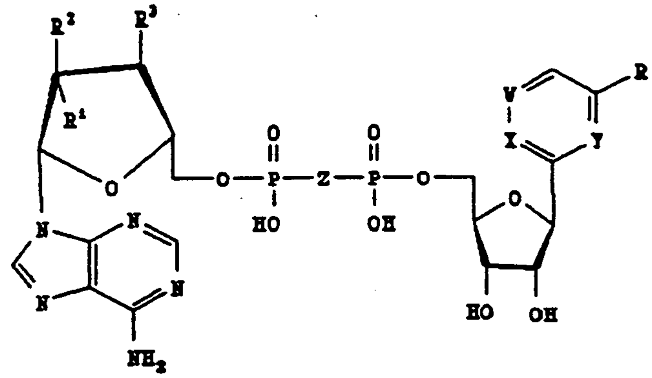
respectively, wherein R, W, X and Y are the same as defined previously and R^1, R^2, R^3 are hydrogen, hydroxy or fluorine;

under suitable conditions to form a compound having the structure:



wherein Z is CH_2 or CF_2 and R, W, X and Y are the same as defined previously and R^1, R^2, R^3 are hydrogen, hydroxy, fluorine or O-protecting groups and B is H or a protecting group; and

e) reacting the compound formed in step (d) under suitable conditions to selectively remove the protecting groups to form the compound having the structure:



wherein Z is CH_2 or CF_2 and R, W, X and Y are the same as defined previously and R^1, R^2, R^3 are hydrogen, hydroxy or fluorine.

In this embodiment, the reaction of step (a) comprises the selective protection of the vicinal cis hydroxy groups on the nucleoside to leave the 5'hydroxyl group as the remaining reactive site. For the purpose of this invention, a "precursor of a suitable protecting group" will comprise any compound that can be reacted with the compound of step (a) to allow for selective replacement of the vicinal hydroxyl cis hydroxy group with the corresponding O-protecting group. Examples of these are well known to those skilled in the art and include, but are not limited to, such compounds as isopropylidene and ethyl orthoformate. In this step the molar ratio of the reactants is in the range of 1:1 to 1:100 and the reaction is carried out at a temperature range of 0 °C to 50 °C for a period of 5 minutes to 2 days.

The reaction of step (b) comprises the replacement of the 5'hydroxy group with a tosyl leaving group by reacting the compound formed in step (a) with tosyl chloride. In this step the molar ratio of the reactants is in the range of 1:1 to 1:10 and the reaction is carried out at a temperature range of -10 °C to 50 °C for a period of 5

minutes to 2 days.

The reaction of step (c) comprises the formation of the methylene- or difluoromethylene-diphosphonate derivative of the compound formed in step (b) by reacting the compound formed in step (b) with tris(tetra-n-butylammonium) methylene diphosphonate or tris(tetra-n-butylammonium) difluoromethylene diphosphonate, respectively, in dimethylsulfoxide. In this step the molar ratio of the reactants is in the range of 1:1 to 1:100 and the reaction is carried out at a temperature range of 0 °C to 50 °C for a period of 5 minutes to 10 days.

The reaction of step (d) comprises the coupling of the compound formed in step (c) with the corresponding nucleoside to form the dinucleotide complexes of this invention after deprotection in step (e). In step (d) the molar ratio of the reactants is in the range of 1:1 to 1:20 and the reaction is carried out at a temperature range of 0 °C to 50 °C for a period of 5 minutes to 10 days. In step (e), the deprotection of the compounds formed in step (d) is carried out at a temperature range of -20 °C to 50 °C for a period of 5 minutes to 1 day.

This invention is further illustrated in the Experimental Details section which follow. The Experimental Details section and Examples contained therein are set forth to aid in an understanding of the invention. This section is not intended to, and should not be interpreted to, limit in any way the invention set forth in the claims which follow thereafter.

Experimental Details

Preparation of the compounds

EXAMPLE 1

5-(β-D-Ribofuranosyl)nicotinamide-(5'-5")-adenosine pyrophosphate. To a suspension of 5-(β-D-ribofuranosyl)nicotinamide (100 mg, 0.4 mmol) in triethylphosphate (0.4 mL) is added phosphoryl chloride (72 mg, 0.48 mmol) at 0° C, and the mixture is stirred at room temperature for 4 hours. The reaction is quenched by addition of water (5 mL), and the mixture is neutralized with concentrated ammonia. The crude product is purified on a column of DEAE Sephadex A-25 (bicarbonate form) with 0.1M tetraethylammonium bicarbonate and then on a Dowex 50W-X8 (H^+) column to give the desired nucleoside 5'-monophosphate (76 mg). This compound is dried by coevaporation with pyridine (3 x 5 mL) and dimethylformamide (3 x 5 mL), and the residue is dissolved in dimethylformamide (0.7 mL). Carbonyldiimidazole (186 mg, 1.15 mmol) is added, and the progress of reaction was followed by thin layer chromatography (iPrOH-conc.NH_4OH-H_2O, 6;3;1, v/v/v). The excess of carbonyldiimidazole is hydrolyzed by addition of methanol (76 μL), and a solution of adenosine 5'-monophosphate (126 mg, 0.35 mmol) in dimethylformamide (4.4 mL) containing tributylamine (80 uL, 0.35 mmol) is added. The reaction mixture is stirred for 3 days. Water (10 mL) is added, and the mixture is concentrated in vacuo. The gummy residue is dissolved in water (40 mL) containing sodium acetate (60 mg) and extracted with chloroform (2 x 40 mL) and diethyl ether (2 x 40 mL). The aqueous layer is treated with triethylamine (60 mL, pH = 10) and then lyophilized. The residue is purified on preparative cellulose plate using iPrOH-conc.NH_4OH-H_2O (6:3:1), and then by a column of Dowex 50W-X8 (H^+) to

give the desired pyrophosphate (90 mg, 60%) as a white powder. ^{1}N NMR (D_2O) δ 4.11-4.41 (m, 8H, H3', H3", H4',H4", H5',H5', H5", H5"), 4.54 (ψt, 1H, H2'), 4.76 (ψt, 1H, H2"), 5.06 (d, 1H, H1', $J_{1'-2'}$ = 7.2 Hz), 6.14 (d, 1H, H1", $J_{1"-2"}$ = 5.2 Hz), 8.43, 8.62 (two 1H singlets, H2, H8), 8.95-9.19 (m, 5H, H2, H4, H6, NH_2). MS (FAB) m/e 662 $(M-H)^-$; 664 MH^+

By following the same procedure, but using the corresponding nucleoside of Formula II instead of nicotinamide riboside, the following dinucleoside pyrophosphates are prepared:

6-(ß-D-Ribofuranosyl)picolinamide-(5'-5")-adenosine pyrophosphate,

2-(ß-D-Ribofuranosyl)isonicotinamide-(5'-5")-adenosine pyrophosphate,

5-(ß-D-Ribofuranosyl)-3-cyanopyridine-(5'-5")-adenosine pyrophosphate,

6-(ß-D-Ribofuranosyl)-2-cyanopyridine-(5'-5")-adenosine pyrophosphate, and

2-(ß-D-Ribofuranosyl)-4-cyanopyridine-(5'-5")-adenosine pyrophosphate.

EXAMPLE 2

6-(ß-D-Ribofuranosyl)picolinamide-(5'-5")-2"-deoxy-2"-fluoroadenosine pyrophosphate (2).

To a suspension of 6-(ß-D-ribofuranosyl)picolinamide (0.195 mmol) in triethylphosphate (0.195 mL) is added phosphoryl chloride (36 mg, 0.24 mmol) at 0°C, the mixture is stirred at room temperature 4 hours, and the diluted with water (5 mL). After addition of concentrated

ammonia, the crude product is purified on a DEAE Sephadex A-25 column (bicarbonate form) with 0.1M tetraethylammonium bicarbonate and Dowex 50W-X8 (H^+ form) to give 5'-monophosphate in 60-70% yield. The compound is then converted into the dinucleotide in reaction with 2'-deoxy-2'-fluoroadenosine 5'-monophosphate as described above. ^{1}H NMR (D_2O) δ 4.15-4.45 (m, 8H, H2', H3', H4', H5', H5', H4", H5", H5"), 4.61 (ddd, 1H, H3", $J_{2",3"}$ = 4.4 Hz, $J_{3",4"}$ = 7.3Hz, $J_{3",P}$ = 20.4 Hz), 5.20 (ddd, 1H, H2", $J_{1",2"}$ = 2.0 Hz, $J_{2",P}$ = 51.9 Hz), 6.25 (dd, 1H, H1", $J_{1",P}$ = 16.0 Hz), 7.61 (dd, H5, $J_{3,5}$ = 1.0 Hz, $J_{4,5}$ = 7.7 Hz), 7.73 (dd, 1H, H3, $J_{3,4}$=7.7 Hz), 7.84 (t, 1H, H4, $J_{3,4}$=$J_{4,5}$=7.7 Hz), 8.11, 8.27 (two 1H singlets, H2, H8), ^{31}P NMR (D_2O) δ -10.6, δ -10.7 ; J_{POP}= 21.0 Hz. MS (FAB) m/e 662 $(M-H)^-$, 664 MH^+

By following the same procedure, but using the corresponding nucleoside of Formula III instead of adenosine, the following dinucleoside pyrophosphates are prepared:

5-(ß-D-Ribofuranosyl)nicotinamide-(5'-5")-2'-deoxyadenosine pyrophosphate,

2-(ß-D-Ribofuranosyl)isonicotinamide-(5'-5")-2'-deoxyadenosine pyrophosphate,

5-(ß-D-Ribofuranosyl)-3-cyanopyridine-(5'-5")-2'-deoxyadenosine pyrophosphate,

6-(ß-D-Ribofuranosyl)-2-cyanopyridine-(5'-5")-2'-deoxyadenosine pyrophosphate,

2-(ß-D-Ribofuranosyl)-4-cyanopyridine-(5'-5")-2'-deoxyadenosine pyrophosphate,

5-(ß-D-Ribofuranosyl)nicotinamide-(5'-5")-2'-deoxy-2'-

fluoroadenosine pyrophosphate,

2-(β-D-Ribofuranosyl)isonicotinamide-(5'-5")-2'-deoxy-2'-fluoroadenosine pyrophosphate,

5-(β-D-Ribofuranosyl)-3-cyanopyridine-(5'-5")-2'-deoxy-2'-fluoroadenosine pyrophosphate,

6-(β-D-Ribofuranosyl)-2-cyanopyridine-(5'-5")-2'-deoxy-2'-fluoroadenosine pyrophosphate,

2-(β-D-Ribofuranosyl)-4-cyanopyridine-(5'-5")-2'-deoxy-2'-fluoroadenosine pyrophosphate,

6-(β-D-Ribofuranosyl)picolinamide-(5'-5")-3'-deoxy-3'-fluoroadenosine pyrophosphate,

2-(β-D-Ribofuranosyl)isonicotinamide-(5'-5")-3'-deoxy-3'-fluoroadenosine pyrophosphate,

5-(β-D-Ribofuranosyl)-3-cyanopyridine-(5'-5")-3'-deoxy-3'-fluoroadenosine pyrophosphate,

6-(β-D-Ribofuranosyl)-2-cyanopyridine-(5'-5")-3'-deoxy-3'-fluoroadenosine pyrophosphate,

2-(β-D-Ribofuranosyl)-4-cyanopyridine-(5'-5")-3'-deoxy-3'-fluoroadenosine pyrophosphate,

6-(β-D-Ribofuranosyl)picolinamide-(5'-5")-9-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)adenine pyrophosphate,

2-(β-D-Ribofuranosyl)isonicotinamide-(5'-5")-9-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)adenine pyrophosphate,

5-(β-D-Ribofuranosyl)-3-cyanopyridine-(5'-5")-9-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)adenine

pyrophosphate,

6-(ß-D-Ribofuranosyl)-2-cyanopyridine-(5′-5″)-9-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)adenine pyrophosphate, and

2-(ß-D-Ribofuranosyl)-4-cyanopyridine-(5′-5″)-9-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)adenine pyrophosphate.

EXAMPLE 3

(5-ß-D-Ribofuranosylnicotiamide-5′-yl)methylenediphosphonate. The 5-ß-D-Ribofuranosylnicotiamide (253 mg, 1mmol) was dissolved in acetone (5 mL), 2,2 -dimethoxypropane (1 mL) and p-toluenosulfonic acid (380, 2mmol) was added and the mixture was stirred at room temperature for 5 h. The mixture was neutralized with $NaHCO_3$, filtered, and concentrated in vacuo. The residue was extracted with chloroform (3 x 5 mL), the organic solution was washed with water (2 x 3 mL) and concentrated in vacuo to give 2′,3′-O-isopropylidene-5-ß-D-ribofuranosylnicotinamide (290 , 99%). ^{1}H NMR ($CDCl_3$) δ 1.32 (s, 3H, iPr), 1.58 (s, 3H, iPr), 3.72-3.95 (m, 2H, H5′, H5″), 4.17-4.21 (m, 1H, H4′), 4.40-4.50 (m, 1H, H3′), 4.71-4.80 (m, 1H, H2′), 4.83 (d, 1H, H1′, $J_{1',2'}$ = 5.3 Hz), 6.90 (brs, 1H, NH_2), 7.56 (brs, 1H, NH_2), 8.19 (s, 1H, H4), 8.60 (s, 1H, H6), 8.86 (s, 1H, H2). The 2′,3′-O-isopropylidene-5-ß-D-ribofuranosylnicotinamide (290 mg, 0.99 mmol) was added into solution of methylene diphosphonate tetrachloride (250 mg, 1 mol) in triethylphospate (5 mL). The mixture was stirred at room temperature for 2h, poured into ice water (10 mL), stirred for 30 min., and then whole mixture was extracted with ethyl acetate (3x10 mL). The pH of the water solution was adjusted to 2 with HCl, the mixture was kept standing for 2 h and concentrated. The

residue was purified on preparative HPLC column (Dynamax-300A C18 83 243 C, rate flow 20 mL/min.) with 0.1 M TEAB followed by linear gradient of 0.1 TEAB /ag. acetonitrile (70%) to give (5-ß-D-ribofuranosylnicotinamide-5'-yl)methylenediphosphonate (440 mg, 72%) as bis triethylammonium salt. ^{1}H NMR (D_2O δ 2.12 (t, 2H, CH_2, $J_{P,H}$ = 20 Hz), 4.05-4.40 (m, 4H, H3', H4', H5', H5"), 4.55 (pseudot, 1H, H2'), 4.79 (d, 1H, H1', $J_{1',2'}$ = 4.9 Hz), 8.40 (s, 1H, H4), 8.72 (s, 1H, H4), 8.91 (s, 1H, H2). ^{31}P NMR (D_2O) δ 11.5 (d, $J_{P,P}$ = 9.5 Hz), 22.8 (d).

By following the same procedure, but using the corresponding nucleoside of Formula II instead of nicotinamide riboside, the following methylenediphosphonates are prepared:

(6-ß-D-Ribofuranosylpicolinamide-5'-yl)methylene-diphosphonate,

(2-ß-D-Ribofuranosylisonicotinamide-5'-yl)methylene-diphosphonate,

(5-ß-D-Ribofuranosyl-3-cyanopyridine-5'-yl)methylene-diphosphonate,

(6-ß-D-Ribofuranosyl-2-cyanopyridine-5'-yl)methylene-diphosphonate, and

(2-(ß-D-Ribofuranosyl-4-cyanopyridine-5'-yl)methylene-diphosphonate.

EXAMPLE 4

(Adenosin-5'-yl)methylenediphosphonate. The 2',3'-O-isopropylidene adenosine (307 mg, 1mmol) was added into solution of methylene diphosphonate tetrachloride (250 mg, 1 mol) in triethylphosphate (5 mL). The mixture was

stirred at room temperature for 2h, poured into ice water (10 mL), stirred for 30 min., and then whole mixture was extracted with ethyl acetate (3x10 mL). The pH of the water solution was adjusted to 2 with HCl, the mixture was kept standing for 2 h and concentrated. The residue was purified on preparative HPLC column (Dynamax-300A C18 83 243 C, rate flow 20 mL/min.) with 0.1 M TEAB followed by linear gradient of 0.1 TEAB /ag. acetonitrile (70%) to give (adenosin-5′-yl)methylenediphosphonate (452 mg, 72%) as bis triethylammonium salt. This compound was identical with the corresponding sample prepared by the reaction of 5′-tosyl adenosine with tris(tetra-n-butylammonium)methylenediphosphonate (Example 6).

By following the same procedure, but using the corresponding nucleoside of Formula III instead of adenosine, the following methylenediphosphonates are prepared:

(2′-deoxyadenosin-5′-yl)methylenediphosphonate,

(2′-deoxy-2′-fluoroadenosin-5′-yl)methylenediphosphonate,

(3′-deoxy-3′-fluoroadenosin-5′-yl)methylenediphosphonate, and

[9-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)adenine-5′-yl]methylenediphosphonate.

EXAMPLE 5

(5-ß-D-Ribofuranosylnicotinamide-5′-yl)difluoromethylenediphosphonate. The 2′,3′-O-isopropylidene-5-ß-D-ribofuranosylnicotinamide (293 mg, 1mmol), obtained as in Example 4, was dissolved in methylene chloride (6 mL), and then dimethylaminopyridine (122 mg, 1 mmol), triethylamine

(202 mg, 2mmol) and tosyl chloride (220, 1.2 mmol) was added. The mixture was stirred for 2h and concentrated in vacuo. The residue was chromatographed on a column of silica gel with chloroform-ethanol (50:1, v/v) as the eluent to give 5'-O-tosyl derivative (410 mg , 92%). ^{1}H NMR ($CDCl_3$) δ 1.34 (s, 3H, iPr), 1.62 (s, 1H, iPr), 2.46 (s, 3H, Ts), 4.21 (dd, 1H, H5', $J_{4',5'}$ = 2.7 Hz, $J_{5',5''}$ = 10.8 Hz), 4.33-4.37 (m, 1H, H4'), 4.43 (dd, 1H, H5", $J_{4',5''}$ = 2.6 Hz), 4.54 (pseudot, 1H, H3'), 4.74 (dd, 1H, H2', $J_{1',2'}$ =5.5 Hz, $J_{2',3'}$= 3.4 Hz), 5.01 (d, 1H, H1'), 5.71 (brs, 1H, NH_2), 6.72 (brs, 1H, NH_2), 7.37 (d, 2H, Ts, J = 8.4 Hz), 7.78 (d, 2H, Ts), 8.25 (s, 1H, H4), 8.68 (brs, 1H, H6), 9.12 (brs, 1H, H2). A solution of 2',3'-O-isopropylidene-5'-O-tosyl-5-ß-D-ribofuranosylnicotinamide (224 mg, 0.5 mmol) and tris(tetra-n-butylammonium)difluoromethylenediphosphonate (700mg, 0.75 mmol) in dimethyl sulfoxide (10 mL) was kept standing for 2h and lyophilized. The residue was dissolved in water and purified on preparative HPLC column as above to give 266 mg, 82% ^{1}H NMR (D_2O) δ 1.42 (s, 3H, iPr), 1.67 (s, 3H, iPr), 4.27-4.33 (m, 2H, H5', H5"), 4.45-4.48 (m, 1H, H4'), 4.80-4.87 (m, 1H, H3'), 5.05-5.12 (m, 2H, H1', H2', $J_{1',2'}$ = 5.7 Hz), 8.42 (s, 1H, H4), 8.73 (s, 1H, H6), 8.92 (s,1H, H2).
^{31}P NMR (D_2), δ 4.12, 7.18 (part AB of ABX_2 system, $J_{A,B}$ = 52.0 Hz, $J_{A,X}$ = 88.5 Hz, $J_{B,X}$ = 73.2 Hz, X = F), ^{19}F NMR (D_2) δ -53.61 (dd, 2F, $J_{F,P}$ = 88.5 Hz, $J_{F,P}$ = 73.2 Hz).

By following the same procedure, but using the corresponding nucleoside of Formula II instead of nicotinamide riboside, the following difluoromethylenediphosphonates are prepared:

(6-ß-D-Ribofuranosylpicolinamide-5'-yl)difluoromethylene-diphosphonate,

(2-ß-D-Ribofuranosylisonicotinamide-5'-yl)-

difluoromethylenediphosphonate,

(5-ß-D-Ribofuranosyl-3-cyanopyridine-5'-yl)-difluoromethylenediphosphonate,

(6-ß-D-Ribofuranosyl-2-cyanopyridine-5'-yl)-difluoromethylenediphosphonate, and

(2-(ß-D-Ribofuranosyl-4-cyanopyridine-5'-yl)-difluoromethylenediphosphonate.

EXAMPLE 6

(Adenosin-5'-yl)difluoromethylenediphosphonate. 5'-O-Tosyladenosine (421, 1mmol) was treated with tris(tertan-butylammonium)difluoromethylenediphosphonate as described in Example 5 to give the (adenosine-5-yl)difluoromethylenediphosphonate in 64% yield.

By following the same procedure, but using the corresponding nucleoside of Formula III instead of adenosine, the following difluoromethylenediphosphonates are prepared:

(2'-deoxyadenosin-5'-yl)difluoromethylenediphosphonate,

(2'-deoxy-2'-fluoroadenosin-5'-yl)difluoromethylene-diphosphonate,

(3'-deoxy-3'-fluoroadenosin-5'-yl)difluoromethylene-diphosphonate, and

[9-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)adenine-5'-yl]difluoromethylenediphosphonate.

EXAMPLE 7

P^1-[5-(ß-D-Ribofuranosyl)nicotinamide-5'-yl]-P^2-[adenosine-5"-yl]methylenediphosphonate and P^1-[(5-ß-D-ribofuranosyl)-3-cyanopyridine-5'-yl]-P^2-[adenosine-5'-yl]methylene-diphosphonate. (Adenosine-5'-yl)methylenediphosphonate (obtained as in Example 4, ..., 1mmol) was dissolved in a mixture of DMSO (20 mL) and triethyl orthoformate (3.9 mL) and trifluoroacetic acid (5 mL) was added. The mixture was stirred for 16 h and lyophilized. The residue was treated with ethyl ether (100 mL). The precipitate was collected by centrifugation and dried at reduced pressure. This product was suspended in pyridine (16 mL) containing tri-n-butyl amine (2.5 mL) and 2',3'-O-isopropylidene-5-ß-D-ribofuranose-nicotinamide (obtained as in Example 3, 322 mg, 1.1 mmol) and dicyclohexylcarbodiimide (DCC, 1.0 g) was added. The reaction was stirred for 4 days and concentrated in vacuo. The residue was suspended in water (100 mL), filtered and the filtrate was treated with Dowex 50W (H^+) for 8 h. The resin was filtered, the filtrate was concentrated in vacuo and the residue was purified on preparative HPLC column as described before to give the ß-methylene CNAD as triethylammonium salt, which was converted to the disodium salt by passing trough Dowex 50W (Na^+) to give (60 mg, 10%). ^1H NMR (D_2O) δ 2.28 (t, 2H, CH_2 $J_{P,H}$= 20.1 Hz), 4.05-4.40 [m, 8H, H3',4',5',5" (adenosine),H3',4',5',5" (nicotinamide riboside)], 4.50 [pseudot, 1H, H2' (NR)], 4.70 [pseudot, 1H, H2'(A)], 4.87 [d, 1H, H1'(NR), $J_{1',2'}$ =4.9 Hz], 6.02 [d, 1H, H1' (A), $J_{1',2'}$ = 5.2 Hz], 8.17 [s, 1H, H4 (NR)], 8.20, 8.45 [two 1H singlets, H2, H8 (A)] , 8.59 [brs, 1H, H6(NR)], 8.75 [brs, 1H, H2 (NR)]. ^{31}P NMR (D_2O) δ 17.63, 17.88 (AB system, $J_{A,B}$ = 10.4 Hz).

Due to dehydratation (DCC) of the desired product, the P^1-[(5-ß-D-ribofuranosyl)-3-cyanopyridine-5'-yl]-P^2-[adenosine-5'-yl]methylenediphosphonate (70 mg 14%) was also obtained. ^1H NMR (D_2O) δ 2.28 (t, 2H, CH_2, $J_{P,H}$ =

20.1 Hz), 4.02-4.42 [m, 8H, H3',4',5',5" (adenosine),H3',4',5',5" (nicotinamide riboside)], 4.55 [pseudot, 1H, H2' (NR)], 4.74 [pseudot, 1H, H2'(A)], 4.79 [d, 1H, H1'(NR), $J_{1',2'}$ = 4.9 Hz], 6.06 [d, 1H, H1' (A), $J_{1',2'}$ = 5.4 Hz], 8.17 [s, 1H, H4 (NR)], 8.20, 8.50 [two 1H singlets, H2, H8 (A)] , 8.69 [s, 1H, H6 (NR)], 8.71 [s, 1H, H2 (NR)]. ^{31}P NMR (D_2O) δ 17.63, 17.88 (AB system, $J_{A,B}$ = 9.9 Hz).

By following the same procedure, but using the corresponding methylenediphosphonates of the compound of Formula III instead of (adenosin-5'-yl)methylene-diphosphonate and nucleosides of Formula II, the following dinucleotides are prepared:

P^1-[6-(ß-D-Ribofuranosyl)picolinamide-5'-yl]-P^2-[adenosine-5"-yl]methylenediphosphonate,

P^1-[2-(ß-D-Ribofuranosyl)isonicotinamide-5'-yl]-P^2-[adenosine-5"-yl]methylenediphosphonate, P^1-[5-(ß-D-Ribofuranosyl)-3-cyanopyridine-5'-yl]-P^2-[adenosine-5"-yl]methylenediphosphonate,

P^1-[6-(ß-D-Ribofuranosyl)-2-cyanopyridine-5'-yl]-P^2-[adenosine-5"-yl]methylenediphosphonate,

P^1-[2-(ß-D-Ribofuranosyl)-4-cyanopyridine-5'-yl]-P^2-[adenosine-5"-yl]methylenediphosphonate.

P^1-[5-(ß-D-Ribofuranosyl)nicotinamide-5'-yl]-P^2-[2'-deoxyadenosine-5"-yl]methylenediphosphonate,

P^1-[6-(ß-D-Ribofuranosyl)picolinamide-5'-yl]-P^2-[2'-deoxyadenosine-5"-yl]methylenediphosphonate,

P^1-[2-(ß-D-Ribofuranosyl)isonicotinamide-5'-yl]-P^2-[2'-deoxyadenosine-5"-yl]methylenediphosphonate,

P^1-[5-(ß-D-Ribofuranosyl)-3-cyanopyridine-5'-yl]P^2-[2'-deoxyadenosine-5"-yl]methylenediphosphonate,

P^1-[6-(ß-D-Ribofuranosyl)-2-cyanopyridine-5'-yl]-P^2-2'-deoxyadenosine-5"-yl]methylenediphosphonate,

P^1-[2-(ß-D-Ribofuranosyl)-4-cyanopyridine-5'-yl]-P^2-[2'-deoxyadenosine-5"-yl]methylenediphosphonate,

P^1-[5-(ß-D-Ribofuranosyl)nicotinamide-5'-yl]-P^2-[2'-deoxy-2'-fluoroadenosine-5"-yl]methylenediphosphonate,

P^1-[6-(ß-D-Ribofuranosyl)picolinamide-5'-yl]-P^2-[2'-deoxy-2'-fluoroadenosine-5"-yl]methylenediphosphonate,

P^1-[2-(ß-D-Ribofuranosyl)isonicotinamide-5'-yl]-P^2-[2'-deoxy-2'-fluoroadenosine-5"-yl]methylenediphosphonate,

P^1-[5-(ß-D-Ribofuranosyl)-3-cyanopyridine-5'-yl]-P^2-[2'-deoxy-2'-fluoroadenosine-5"-yl]methylenediphosphonate,

P^1-[6-(ß-D-Ribofuranosyl)-2-cyanopyridine-5'-yl]-P^2-[2'-deoxy-2'-fluoroadenosine-5"-yl]methylenediphosphonate,

P^1-[2-(ß-D-Ribofuranosyl)-4-cyanopyridine-5'-yl]-P^2-[2'-deoxy-2'-fluoroadenosine-5"-yl]methylenediphosphonate,

P^1-[5-(ß-D-Ribofuranosyl)nicotinamide-5'-yl]-P^2-[3'-deoxy-3'-fluoroadenosine-5"-yl]methylenediphosphonate,

P^1-[6-(ß-D-Ribofuranosyl)picolinamide-5'-yl]-P^2-[3'-deoxy-3'-fluoroadenosine-5"-yl]methylenediphosphonate,

P^1-[(ß-D-Ribofuranosyl)isonicotinamide-5'-yl]-P^2-[3'-deoxy-3'-fluoroadenosine-5"-yl]methylenediphosphonate,

P^1-[5-(ß-D-Ribofuranosyl)-3-cyanopyridine-5'-yl]-P^2-[3'-

deoxy-3′-fluoroadenosine-5″-yl]methylenediphosphonate,

P[1]-[6-(ß-D-Ribofuranosyl)-2-cyanopyridine-5′-yl]-P[2]-[3′-deoxy-3′-fluoroadenosine-5″-yl]methylenediphosphonate,

P[1]-[2-(ß-D-Ribofuranosyl)-4-cyanopyridine-5′-yl]-P[2]-[3′-deoxy-3′-fluoroadenosine-5″-yl]methylenediphosphonate,

P[1]-[5-(ß-D-Ribofuranosyl)nicotinamide-5′-yl]-P[2]-[9-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)adenine-5″-yl]-methylenediphosphonate,

P[1]-[6-(ß-D-Ribofuranosyl)picolinamide-5′-yl]-P[2]-[9-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)adenine-5″-yl]-methylenediphosphonate,

P[1]-[2-(ß-D-Ribofuranosyl)isonicotinamide-5′-yl]-P[2]-[9-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)adenine-5″-yl]-methylenediphosphonate,

P[1]-[5-(ß-D-Ribofuranosyl)-3-cyanopyridine-5′-yl]-P[2]-[9-(2′-deoxy-2′-fluoro-β-D- arabinofuranosyl)adenine-5″-yl]-methylenediphosphonate,

P[1]-[6-(ß-D-Ribofuranosyl)-2-cyanopyridine-5′-yl]-P[2]-[9-(2′-deoxy-2′-fluoro-β-D- arabinofuranosyl)adenine-5″-yl]-methylenediphosphonate, and

P[1]-[2-(ß-D-Ribofuranosyl)-4-cyanopyridine-5′-yl]-P[2]-[9-(2′-deoxy-2′-fluoro-β-D- arabinofuranosyl)adenine-5″-yl]-methylenediphosphonate.

EXAMPLE 8

P[1]-[5-(ß-D-Ribofuranosyl)nicotinamide-5′-yl]-P[2]-[adenosine-5″-yl]difluoromethylenedi-phosphonate. The (2′,3′-O-isopropylidene-5-ß-D-ribofuranosylnicotinamide-

5'-yl)difluoromethylenediphosphonate (obtained as in Example 5, 649 mg, 1mmol) was coupled with 2',3'-O-isopropylidene adenosine (4.6g, 15 mmol) in the same manner as above to give ß-difluoromethylene CNAD (50, 7%) ^{1}H NMR (D_2O) 4.05-4.40 (m, 8H, H3', H4', H5', H5" -A and NR), 4.53 (pseudot, 1H, H2' (NR), 468 (pseudot, 1H (A), 4.90 (d, 1H, H1' (NR), $J_{1',2'}$ = 5.1 Hz), 5.99 (d, 1H, H1' (A), $J_{1',2'}$ = 5.3 Hz), 8.21 (s, 1H, H4 (NR)], 8.26, 8.50 [two 1H singlets, H2, H8 (A)], 8.61 (s, 1H, H6 (NR)], 8.80 (s, 1H, H2 (NR)].

^{31}P NMR (D_2O) δ 4.02, 4.36 (AB part of ABX_2 system, $J_{A,B}$ = 55.3 Hz, $J_{A,X}$ =83.1 Hz, $J_{B,X}$ = 83.5 Hz, X = F).

By following the same procedure, but using the corresponding difluoromethylenediphosphonate derivative of the compound of Formula II instead of (5-ß-D-ribofuranosylnicotinamide-5'-yl)difluoromethylene-diphosphonate and nucleosides of Formula III, the following dinucleotides are prepared:

P^1-[6-(ß-D-Ribofuranosyl)picolinamide-5'-yl]-P^2-[adenosine-5"-yl]difluoromethylenediphosphonate,

P^1-[2-(ß-D-Ribofuranosyl)isonicotinamide-5'-yl]-P^2-[adenosine-5"-yl]difluoromethylenediphosphonate,

P^1-[5-(ß-D-Ribofuranosyl)-3-cyanopyridine-5'-yl]-P^2-[adenosine-5"-yl]difluoromethylenediphosphonate,

P^1-[6-(ß-D-Ribofuranosyl)-2-cyanopyridine-5'-yl]-P^2-[adenosine-5"-yl]difluoromethylenediphosphonate,

P^1-[2-(ß-D-Ribofuranosyl)-4-cyanopyridine-5'-yl]-P^2-[adenosine-5"-yl]difluoromethylenediphosphonate,

P^1-[5-(ß-D-Ribofuranosyl)nicotinamide-5'-yl]-P^2-[2'-deoxyadenosine-5"-yl]difluoromethylenediphosphonate,

P^1-[6-(ß-D-Ribofuranosyl)picolinamide-5'-yl]-P^2-[2'-deoxyadenosine-5"-yl]difluoromethylenediphosphonate,

P^1-[2-(ß-D-Ribofuranosyl)isonicotinamide-5'-yl]-P^2-[2'-deoxyadenosine-5"-yl]difluoromethylenediphosphonate,

P^1-[5-(ß-D-Ribofuranosyl)-3-cyanopyridine-5'-yl]P^2-[2'-deoxyadenosine-5"-yl]difluoromethylenediphosphonate,

P^1-[6-(ß-D-Ribofuranosyl)-2-cyanopyridine-5'-yl]-P^2-2'-deoxyadenosine-5"-yl]difluoromethylenediphosphonate,

P^1-[2-(ß-D-Ribofuranosyl)-4-cyanopyridine-5'-yl]-P^2-[2'-deoxyadenosine-5"-yl]difluoromethylenediphosphonate,

P^1-[5-(ß-D-Ribofuranosyl)nicotinamide-5'-yl]-P^2-[2'-deoxy-2'-fluoroadenosine-5"-yl]difluoromethylenediphosphonate,

P^1-[6-(ß-D-Ribofuranosyl)picolinamide-5'-yl]-P^2-[2'-deoxy-2'-fluoroadenosine-5"-yl]difluoromethylenediphosphonate,

P^1-[2-(ß-D-Ribofuranosyl)isonicotinamide-5'-yl]-P^2-[2'-deoxy-2'-fluoroadenosine-5"-yl]difluoromethylenediphosphonate,

P^1-[5-(ß-D-Ribofuranosyl)-3-cyanopyridine-5'-yl]-P^2-[2'-deoxy-2'-fluoroadenosine-5"-yl]difluoromethylenediphosphonate,

P^1-[6-(ß-D-Ribofuranosyl)-2-cyanopyridine-5'-yl]-P^2-[2'-deoxy-2'-fluoroadenosine-5"-yl]difluoromethylenediphosphonate,

P^1-[2-(ß-D-Ribofuranosyl)-4-cyanopyridine-5'-yl]-P^2-[2'-deoxy-2'-fluoroadenosine-5"-yl]difluoromethylenediphosphonate,

P[1]-[5-(ß-D-Ribofuranosyl)nicotinamide-5'-yl]-P[2]-[3'-deoxy-3'-fluoroadenosine-5"-yl]difluoromethylenediphosphonate,

P[1]-[6-(ß-D-Ribofuranosyl)picolinamide-5'-yl]-P[2]-[3'-deoxy-3'-fluoroadenosine-5"-yl]difluoromethylenediphosphonate,

P[1]-(ß-D-Ribofuranosyl)isonicotinamide-5'-yl-P[2]-3'-deoxy-3'-fluoroadenosine-5"-yl-fluoro]difluoromethylenediphosphonate,

P[1]-[5-(ß-D-Ribofuranosyl)-3-cyanopyridine-5'-yl]-P[2]-[3'-deoxy-3'-fluoroadenosine-5"-yl]difluoromethylenediphosphonate,

P[1]-[6-(ß-D-Ribofuranosyl)-2-cyanopyridine-5'-yl]-P[2]-[3'-deoxy-3'-fluoroadenosine-5"-yl]difluoromethylenediphosphonate,

P[1]-[2-(ß-D-Ribofuranosyl)-4-cyanopyridine-5'-yl]-P[2]-[3'-deoxy-3'-fluoroadenosine-5"-yl]difluoromethylenediphosphonate,

P[1]-[5-(ß-D-Ribofuranosyl)nicotinamide-5'-yl]-P[2]-[9-(2'-deoxy-2'-fluoro-β-D-arabino-furanosyl)]adenine-5"-yl]difluoromethylenediphosphonate,

P[1]-[6-(ß-D-Ribofuranosyl)picolinamide-5'-yl]-P[2]-[9-(2'-deoxy-2'-fluoro-β-D-arabino-furanosyl)]adenine-5"-yl]difluoromethylenediphosphonate,

P[1]-[2-(ß-D-Ribofuranosyl)isonicotinamide-5'-yl]-P[2]-[9-(2'-deoxy-2'-fluoro-β-D-arabino-furanosyl)adenine-5"-yl]difluoromethylenediphosphonate,

P[1]-[5-(ß-D-Ribofuranosyl)-3-cyanopyridine-5'-yl]-P[2]-[9-(2'-deoxy-2'-fluoro-β-D-arabino-furanosyl)adenine-5"-yl]difluoromethylenediphosphonate,

P^1-[6-(ß-D-Ribofuranosyl)-2-cyanopyridine-5′-yl]-P^2-[9-(2′-deoxy-2′-fluoro-β-D-arabino-furanosyl)adenine-5"-yl]difluoromethylenediphosphonate, and

P^1-[2-(ß-D-Ribofuranosyl)-4-cyanopyridine-5′-yl]-P^2-[9-(2′-deoxy-2′-fluoro-β-D-arabino-furanosyl)adenine-5"-yl]difluoromethylenediphosphonate.

Biological Activity

1. Cytotoxicity of 5-(ß-D-ribofuranosyl)nicotinamide-(5′-5")-adenosine pyrophosphate (2, CNAD) to murine leukemia L1210 cells.

Murine leukemia L1210 cells were grown in RPMI 1640 medium. Logarithmically growing cells were incubated with various concentrations of CNAD for 24 and 48 hr periods and the cytotoxicity determined by counting the cells in a Coulter counter.

It was found that the 5-(ß-D-ribofuranosyl)nicotinamide-(5′-5")-adenosine pyrophosphate (2, CNAD) inhibits the proliferation of L1210 cells by 50% (IC_{50}) at the concentration of 7 μM.

2. Inhibition of horse liver alcohol dehydrogenase (ADH) and bovine glutamate dehydrogenase (GDH)

Rate measurements for each of the dehydrogenases used in this study are based on the spectral properties of NADH. In assays with NAD as a substrate, rates were determined by measuring the increase in absorbance at 340 nm resulting from the conversion of NAD to NADH. Rates, using absorbance measurements, were calculated using a millimolar extinction coefficient of 6.22/cm for NADH.

Alcohol dehydrogenase assays were run at pH 8.0, using 0.1M sodium phosphate buffer. Glutamate dehydrogenase assays were at pH 7.0 in 0.1M sodium phosphate buffer, containing 10 μm EDTA. All kinetic assays were run at least in duplicate.

Initial values of inhibition constants were estimated from Lineweaver-Burk plots, using linear regression to obtain values for the slope and intercept of each line. Inhibition was judged to be competitive if the values obtained for the intercepts of the appropriate polots differed by less than three standard deviations as determined by linear regression. Values for the inhibition constant, K_{is} was obtained using the relationship:

$$K_{is} = [I]/\{[S(+)/S(-)]-1\}$$

where S(+) and S(-) are the slopes of the plots in the presence and absence of inhibitor, respectively, and [I] is the concentration of total added inhibitor. Where a noncompetitive pattern of inhibition was observed, a similar analysis was used to obtain K_{ii}. K_m for conenzyme was obtained from the slope and intercept obtained in plots in the absence of inhibitor, using the relationship: K_m = Slope/V_{max}.

Values of effective inhibition constants and patterns of inhibition for CNAD binding to ADH were obtained by direct least-squares fits to the nonreciprocal forms of the Michaelis-Menten rate equations. Kinetic data were fit to the following relationships, assuming both competitive and noncompetitive inhibition respectively:

$$v_o = V_m [A]/\{K_m(1+[I]/K_{is})+[A]\} \quad \text{or}$$

$$v_o = V_m [A]/\{K_m(1+[I]/K_{is})+[A](1+[I]/K_{ii})\}$$

Where v_o is the initial reaction rate, V_m is the maximal rate, and K_m and [A] the Michaelis constant and concentration of the variable substrate, respectively. The pattern of inhibition considered to best account for the observed data was that giving both the smallest residuals between observed and calculated values, and the smallest standard errors in the computed kinetics constants. This method demonstrated non-competitive inhibition of ADH with respect to NAD by CNAD with an apparent K_i ($K_{i'}$) of 6 nM.

In order to estimate the magnitude of nonlinear effects introduced by tight binding by CNAD, apparent K_i's with respect to NAD were also obtained by a fit of kinetic data to Sculley and Morrison's nonlinear rate equation:

$$v_o = \frac{k_{cat}[A]}{2(Km + [A])}\left[\{([E]-[I]-K_i)^2 + 4K_i\}^{1/2} - (K_i+[I]-[E])\right]$$

where [E] is the total enzyme concentration, k_{cat} is the maximum rate of product formation and, in this case, [A] is the concentration and Km the Michaelis constant of NAD. Derivation of this rate equation assumes the presence of a tight binding inhibitor, i.e., that [I] ~ [E].

In this experiment both inhibitor and enzyme concentration were varied. Initial rates v_o were measured at total concentrations of CNAD ([I]) of 0, 2.4, 9.6, and 19.2 nM over four concentrations of ADH ([E]). Concentrations of ehtanol and NAD were fixed at 1.2nM and 87 μM at pH 8.0. The apparent inhibition constant with respect to NAD, $K_{i'}$, was then obtained by non-linear least-squares fit to the rate equation under the array of experimental conditions employed. The true rate constant K_i was obtained from the apparent rate constant K_i was

obtained from the apparent rate constant $K_{i'}$ via the relationship:

$$K_i = K_{i'}/(1+[A]/K_m)$$

Results for 6-(β-D-ribofuranosyl)picolinamide-(5'-5")-adenosine pyrophosphate (**C-PAD**) indicate competitive inhibition of ADH with respect to NAD, with K_i = 20 μM. The results for **CNAD**, however, showed competitive inhibition of GDH (K_i = 15 μM), but non-competitive inhibition of ADH, with K_i = 2 nM.

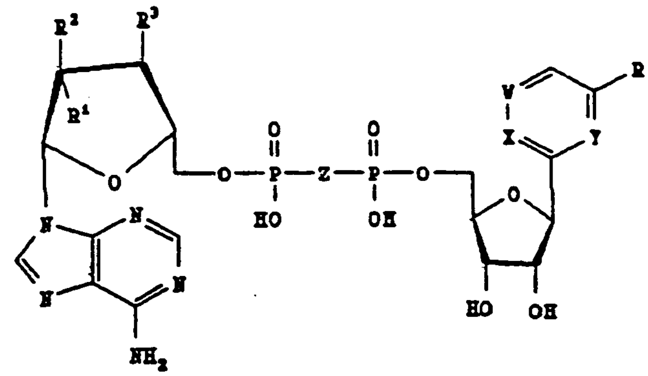
Discussion

Inhibition of alcohol dehydrogenase (ADH) provides potential therapies for ethylene glycol intoxication, ethanol-induced hypoglycemia and lactacidemia and methanol poisoning. This along with the extensive structural information available about the enzyme and its complexes, have made ADH an attractive target for inhibitor design. A number of classes of highly potent *reversible* ADH-inhibitors have been developed. These are 4-substituted alkylpyrazoles, 1-mercapto-n-alkanes, phenylacetamide and formamide derivatives and adloximes. Like **CNAD**, these inhibitors bind the catalytic site Zn via a nitrogen, oxygen or sulfur ligand. Unlike **CNAD**, these compounds act as substrate analogues, binding Zn from the substrate site, with alkyl or phenyl groups extending into the hydrophobic substrate cleft. Inhibitors of this type can bind in-ternary complexes with cofactor, forming a secondary ligand to the nicotinamide ring. NAD analogues have been developed as inactivating affinity labels, forming covalent interactions with active site residues. However, **CNAD** is the first cofactor analogue which reversibly interacts with the catalytic Zn.

Similar selectivity for these compounds is asserted for inosine monophosphate dehydrogenase (IMPDH). As a result, such NAD analogues should be valuable in cancer treatment. NAD-analogues, not nucleosides, that are able to penetrate the cell membrane may be of therapeutic interest since nucleosides related to nicotinamide riboside are not effectively metabolized into their corresponding NAD-analogues. They do not require metabolic activation by cellular enzymes.

What is claimed is:

1. A compound having the structure:



wherein R^1, R^2, and R^3 are same or different, and are hydrogen, hydroxyl, or fluorine; Z is O, CH_2 or CF_2; R is chlorine, bromine, iodine, carbonitrile, carboxylic ester, or carboxamide; and one of W, X and Y is N or N^+R', wherein R' is methyl or ethyl, and all others are CH; provided that R^1 is not H when R^2 and R^3 are OH, Z is O, R is carboxamide and W is N or N^+R'.

2. A compound of claim 1 selected from the group consisting of:

5-(ß-D-Ribofuranosyl)nicotinamide-(5'-5")-adenosine pyrophosphate,

6-(ß-D-Ribofuranosyl)picolinamide-(5'-5")-adenosine pyrophosphate,

2-(ß-D-Ribofuranosyl)isonicotinamide-(5'-5")-adenosine pyrophosphate,

5-(ß-D-Ribofuranosyl)-3-cyanopyridine-(5'-5")-adenosine pyrophosphate,

6-(ß-D-Ribofuranosyl)-2-cyanopyridine-(5'-5")-adenosine pyrophosphate,

2-(ß-D-Ribofuranosyl)-4-cyanopyridine-(5'-5")-adenosine pyrophosphate,

5-(ß-D-Ribofuranosyl)nicotinamide-(5'-5")-2'-deoxyadenosine pyrophosphate,

6-(ß-D-Ribofuranosyl)picolinamide-(5'-5")-2'-deoxyadenosine pyrophosphate,

2-(ß-D-Ribofuranosyl)isonicotinamide-(5'-5")-2'-deoxyadenosine pyrophosphate,

5-(ß-D-Ribofuranosyl)-3-cyanopyridine-(5'-5")-2'-deoxyadenosine pyrophosphate,

6-(ß-D-Ribofuranosyl)-2-cyanopyridine-(5'-5")-2'-deoxyadenosine pyrophosphate,

2-(ß-D-Ribofuranosyl)-4-cyanopyridine-(5'-5")-2'-deoxyadenosine pyrophosphate,

5-(ß-D-Ribofuranosyl)nicotinamide-(5'-5")-2'-deoxy-2'-fluoroadenosine pyrophosphate,

6-(ß-D-Ribofuranosyl)picolinamide-(5'-5")-2"-deoxy-2"-fluoroadenosine pyrophosphate,

2-(ß-D-Ribofuranosyl)isonicotinamide-(5'-5")-2'-deoxy-2'-fluoroadenosine pyrophosphate,

5-(ß-D-Ribofuranosyl)-3-cyanopyridine-(5'-5")-2'-deoxy-2'-fluoroadenosine pyrophosphate,

6-(ß-D-Ribofuranosyl)-2-cyanopyridine-(5'-5")-2'-

deoxy-2'-fluoroadenosine pyrophosphate,

2-(β-D-Ribofuranosyl)-4-cyanopyridine-(5'-5")-2'-deoxy-2'-fluoroadenosine pyrophosphate,

5-(β-D-Ribofuranosyl)nicotinamide-(5'-5")-3'-deoxy-3'-fluoroadenosine pyrophosphate,

6-(β-D-Ribofuranosyl)picolinamide-(5'-5")-3'-deoxy-3'-fluoroadenosine pyrophosphate,

2-(β-D-Ribofuranosyl)isonicotinamide-(5'-5")-3'-deoxy-3'-fluoroadenosine pyrophosphate,

5-(β-D-Ribofuranosyl)-3-cyanopyridine-(5'-5")-3'-deoxy-3'-fluoroadenosine pyrophosphate,

6-(β-D-Ribofuranosyl)-2-cyanopyridine-(5'-5")-3'-deoxy-3'-fluoroadenosine pyrophosphate,

2-(β-D-Ribofuranosyl)-4-cyanopyridine-(5'-5")-3'-deoxy-3'-fluoroadenosine pyrophosphate,

5-(β-D-Ribofuranosyl)nicotinamide-(5'-5")-9-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)-adenine pyrophosphate,

6-(β-D-Ribofuranosyl)picolinamide-(5'-5")-9-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)-adenine pyrophosphate,

2-(β-D-Ribofuranosyl)isonicotinamide-(5'-5")-9-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)-adenine pyrophosphate,

5-(β-D-Ribofuranosyl)-3-cyanopyridine-(5'-5")-9-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)-adenine

pyrophosphate,

6-(ß-D-Ribofuranosyl)-2-cyanopyridine-(5'-5")-9-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)-adenine pyrophosphate, and

2-(ß-D-Ribofuranosyl)-4-cyanopyridine-(5'-5")-9-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)-adenine pyrophosphate.

3. A compound of claim 1 selected from the group consisting of:

P^1-[5-(ß-D-Ribofuranosyl)nicotinamide-5'-yl]-P^2-[adenosine-5"-yl]methylenediphosphonate,

P^1-[6-(ß-D-Ribofuranosyl)picolinamide-5'-yl]-P^2-[adenosine-5"-yl]methylenediphosphonate,

P^1-[2-(ß-D-Ribofuranosyl)isonicotinamide-5'-yl]-P^2-[adenosine-5"-yl]methylenediphosphonate,

P^1-[5-(ß-D-Ribofuranosyl)-3-cyanopyridine-5'-yl]-P^2-[adenosine-5"-yl]methylenediphosphonate,

P^1-[6-(ß-D-Ribofuranosyl)-2-cyanopyridine-5'-yl]-P^2-[adenosine-5"-yl]methylenediphosphonate,

P^1-[2-(ß-D-Ribofuranosyl)-4-cyanopyridine-5'-yl]-P^2-[adenosine-5"-yl]methylenediphosphonate,

P^1-[5-(ß-D-Ribofuranosyl)nicotinamide-5'-yl]-P^2-[2'-deoxyadenosine-5"-yl]methylenediphosphonate,

P^1-[6-(ß-D-Ribofuranosyl)picolinamide-5'-yl]-P^2-[2'-deoxyadenosine-5"-yl]methylenediphosphonate,

P^1-[2-(ß-D-Ribofuranosyl)isonicotinamide-5'-yl]-P^2-[2'-deoxyadenosine-5"-yl]methylenediphosphonate,

P^1-[5-(ß-D-Ribofuranosyl)-3-cyanopyridine-5'-yl]P^2-[2'-deoxyadenosine-5"-yl]methylenediphosphonate,

P^1-[6-(ß-D-Ribofuranosyl)-2-cyanopyridine-5'-yl]-P^2-2'-deoxyadenosine-5"-yl]methylenediphosphonate,

P^1-[2-(ß-D-Ribofuranosyl)-4-cyanopyridine-5'-yl]-P^2-[2'-deoxyadenosine-5"-yl]methylenediphosphonate,

P^1-[5-(ß-D-Ribofuranosyl)nicotinamide-5'-yl]-P^2-[2'-deoxy-2'-fluoroadenosine-5"-yl]-methylenediphosphonate,

P^1-[6-(ß-D-Ribofuranosyl)picolinamide-5'-yl]-P^2-[2'-deoxy-2'-fluoroadenosine-5"-yl]-methylenediphosphonate,

P^1-[2-(ß-D-Ribofuranosyl)isonicotinamide-5'-yl]-P^2-[2'-deoxy-2'-fluoroadenosine-5"-yl]-methylenediphosphonate,

P^1-[5-(ß-D-Ribofuranosyl)-3-cyanopyridine-5'-yl]-P^2-[2'-deoxy-2'-fluoroadenosine-5"-yl]-methylenediphosphonate,

P^1-[6-(ß-D-Ribofuranosyl)-2-cyanopyridine-5'-yl]-P^2-[2'-deoxy-2'-fluoroadenosine-5"-yl]-methylenediphosphonate,

P^1-[2-(ß-D-Ribofuranosyl)-4-cyanopyridine-5'-yl]-P^2-[2'-deoxy-2'-fluoroadenosine-5"-yl]-methylenediphosphonate,

P^1-[5-(ß-D-Ribofuranosyl)nicotinamide-5'-yl]-P^2-[3'-

deoxy-3'-fluoroadenosine-5"-yl]-methylenediphosphonate,

P[1]-[6-(β-D-Ribofuranosyl)picolinamide-5'-yl]-P[2]-[3'-deoxy-3'-fluoroadenosine-5"-yl]-methylenediphosphonate,

P[1]-[2-(β-D-Ribofuranosyl)isonicotinamide-5'-yl]-P[2]-[3'-deoxy-3'-fluoroadenosine-5"-yl]-methylenediphosphonate,

P[1]-[5-(β-D-Ribofuranosyl)-3-cyanopyridine-5'-yl]-P[2]-[3'-deoxy-3'-fluoroadenosine-5"-yl]-methylenediphosphonate,

P[1]-[6-(β-D-Ribofuranosyl)-2-cyanopyridine-5'-yl]-P[2]-[3'-deoxy-3'-fluoroadenosine-5"-yl]-methylenediphosphonate,

P[1]-[2-(β-D-Ribofuranosyl)-4-cyanopyridine-5'-yl]-P[2]-[3'-deoxy-3'-fluoroadenosine-5"-yl]-methylenediphosphonate,

P[1]-[5-(β-D-Ribofuranosyl)nicotinamide-5'-yl]-P[2]-[9-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)adenine-5"-yl]methylenediphosphonate,

P[1]-[6-(β-D-Ribofuranosyl)picolinamide-5'-yl]-P[2]-[9-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)adenine-5"-yl]methylenediphosphonate,

P[1]-[2-(β-D-Ribofuranosyl)isonicotinamide-5'-yl]-P[2]-[9-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)adenine-5"-yl]methylenediphosphonate,

P[1]-[5-(β-D-Ribofuranosyl)-3-cyanopyridine-5'-yl]-P[2]-[9-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)adenine-

5"-yl]methylenediphosphonate,

P^1-[6-(β-D-Ribofuranosyl)-2-cyanopyridine-5'-yl]-P^2-[9-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)adenine-5"-yl]methylenediphosphonate,

P^1-[2-(β-D-Ribofuranosyl)-4-cyanopyridine-5'-yl]-P^2-[9-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)-adenine-5"-yl]methylenediphosphonate,

P^1-[5-(β-D-Ribofuranosyl)nicotinamide-5'-yl]-P^2-[adenosine-5"-yl]difluoromethylenediphosphonate,

P^1-[6-(β-D-Ribofuranosyl)picolinamide-5'-yl]-P^2-[adenosine-5"-yl]difluoromethylenediphosphonate,

P^1-[2-(β-D-Ribofuranosyl)isonicotinamide-5'-yl]-P^2-[adenosine-5"-yl]difluoromethylenediphosphonate,

P^1-[5-(β-D-Ribofuranosyl)-3-cyanopyridine-5'-yl]-P^2-[adenosine-5"-yl]difluoromethylenediphosphonate,

P^1-[6-(β-D-Ribofuranosyl)-2-cyanopyridine-5'-yl]-P^2-[adenosine-5"-yl]difluoromethylenediphosphonate,

P^1-[2-(β-D-Ribofuranosyl)-4-cyanopyridine-5'-yl]-P^2-[adenosine-5"-yl]difluoromethylenediphosphonate,

P^1-[5-(β-D-Ribofuranosyl)nicotinamide-5'-yl]-P^2-[2'-deoxyadenosine-5"-yl]difluoromethylenediphosphonate,

P^1-[6-(β-D-Ribofuranosyl)picolinamide-5'-yl]-P^2-[2'-deoxyadenosine-5"-yl]difluoromethylenediphosphonate,

P^1-[2-(β-D-Ribofuranosyl)isonicotinamide-5'-yl]-P^2-

[2'-deoxyadenosine-5"-yl]-difluoromethylenediphosphonate,

P[1]-[5-(ß-D-Ribofuranosyl)-3-cyanopyridine-5'-yl]P[2]-[2'-deoxyadenosine-5"-yl]difluoromethylenediphosphonate,

P[1]-[6-(ß-D-Ribofuranosyl)-2-cyanopyridine-5'-yl]-P[2]-2'-deoxyadenosine-5"-yl]difluoromethylenediphosphonate,

P[1]-[2-(ß-D-Ribofuranosyl)-4-cyanopyridine-5'-yl]-P[2]-[2'-deoxyadenosine-5"-yl]difluoromethylenediphosphonate,

P[1]-[5-(ß-D-Ribofuranosyl)nicotinamide-5'-yl]-P[2]-[2'-deoxy-2'-fluoroadenosine-5"-yl]difluoromethylenediphosphonate,

P[1]-[6-(ß-D-Ribofuranosyl)picolinamide-5'-yl]-P[2]-[2'-deoxy-2'-fluoroadenosine-5"-yl]difluoromethylenediphosphonate,

P[1]-[2-(ß-D-Ribofuranosyl)isonicotinamide-5'-yl]-P[2]-[2'-deoxy-2'-fluoroadenosine-5"-yl]-difluoromethylenediphosphonate,

P[1]-[5-(ß-D-Ribofuranosyl)-3-cyanopyridine-5'-yl]-P[2]-[2'-deoxy-2'-fluoroadenosine-5"-yl]-difluoromethylenediphosphonate,

P[1]-[6-(ß-D-Ribofuranosyl)-2-cyanopyridine-5'-yl]-P[2]-[2'-deoxy-2'-fluoroadenosine-5"-yl]-difluoromethylenediphosphonate,

P[1]-[2-(ß-D-Ribofuranosyl)-4-cyanopyridine-5'-yl]-P[2]-[2'-deoxy-2'-fluoroadenosine-5"-yl]-

difluoromethylenediphosphonate,

P^1-[5-(ß-D-Ribofuranosyl)nicotinamide-5'-yl]-P^2-[3'-deoxy-3'-fluoroadenosine-5"-yl]-difluoromethylenediphosphonate,

P^1-[6-(ß-D-Ribofuranosyl)picolinamide-5'-yl]-P^2-[3'-deoxy-3'-fluoroadenosine-5"-yl]-difluoromethylenediphosphonate,

P^1-(2-ß-D-Ribofuranosyl)isonicotinamide-5'-yl-P^2-3'-deoxy-3'-fluoroadenosine-5"-yl]-difluoromethylenediphosphonate,

P^1-[5-(ß-D-Ribofuranosyl)-3-cyanopyridine-5'-yl]-P^2-[3'-deoxy-3'-fluoroadenosine-5"-yl]-difluoromethylenediphosphonate,

P^1-[6-(ß-D-Ribofuranosyl)-2-cyanopyridine-5'-yl]-P^2-[3'-deoxy-3'-fluoroadenosine-5"-yl]-difluoromethylenediphosphonate,

P^1-[2-(ß-D-Ribofuranosyl)-4-cyanopyridine-5'-yl]-P^2-[3'-deoxy-3'-fluoroadenosine-5"-yl]-difluoromethylenediphosphonate,

P^1-[5-(ß-D-Ribofuranosyl)nicotinamide-5'-yl]-P^2-[9-(2'-deoxy-2'-fluoro-β-D-arabino-furanosyl)]adenine-5"-yl]-difluoromethylenediphosphonate,

P^1-[6-(ß-D-Ribofuranosyl)picolinamide-5'-yl]-P^2-[9-(2'-deoxy-2'-fluoro-β-D-arabino-furanosyl)]adenine-5"-yl]-difluoromethylenediphosphonate,

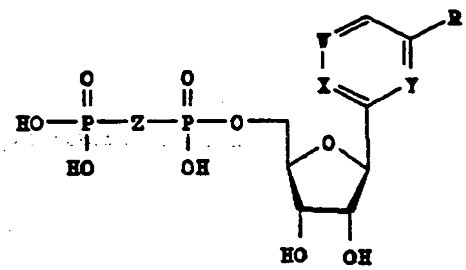
P^1-[2-(ß-D-Ribofuranosyl)isonicotinamide-5'-yl]-P^2-[9-(2'-deoxy-2'-fluoro-β-D-arabino-furanosyl)-adenine-5"-yl]-difluoromethylenediphosphonate,

P^1-[5-(ß-D-Ribofuranosyl)-3-cyanopyridine-5'-yl]-P^2-[9-(2'-deoxy-2'-fluoro-β-D-arabino-furanosyl)-adenine-5"-yl]-difluoromethylenediphosphonate,

P^1-[6-(ß-D-Ribofuranosyl)-2-cyanopyridine-5'-yl]-P^2-[9-(2'-deoxy-2'-fluoro-β-D-arabino-furanosyl)-adenine-5"-yl]-difluoromethylenediphosphonate, and

P^1-[2-(ß-D-Ribofuranosyl)-4-cyanopyridine-5'-yl]-P^2-[9-(2'-deoxy-2'-fluoro-β-D-arabino-furanosyl)-adenine-5"-yl]-difluoromethylenediphosphonate.

4. A compound having the structure:



wherein Z is CH_2 or CF_2; R is chlorine, bromine, iodine, carbonitrile, carboxylic ester, or carboxamide; and one of W, X and Y is N or N^+R', wherein R' is methyl or ethyl, and all others are CH.

5. A compound of claim 4 selected from the group consisting of:

(5-ß-D-Ribofuranosylnicotiamide-5'-yl)methylenediphosphonate

(6-ß-D-Ribofuranosylpicolinamide-5'-yl)methylenediphosphonate,
(2-ß-D-Ribofuranosylisonicotinamide-5'-yl)methylene-diphosphonate,

(5-ß-D-Ribofuranosyl-3-cyanopyridine-5'-yl)methylene-diphosphonate,

(6-ß-D-Ribofuranosyl-2-cyanopyridine-5'-yl)methylene-diphosphonate,

(2-(ß-D-Ribofuranosyl-4-cyanopyridine-5'-yl)methylene-diphosphonate,

(5-ß-D-Ribofuranosylnicotinamide-5'-yl)difluoromethylene-diphosphonate,

(6-ß-D-Ribofuranosylpicolinamide-5'-yl)difluoromethylene-diphosphonate,

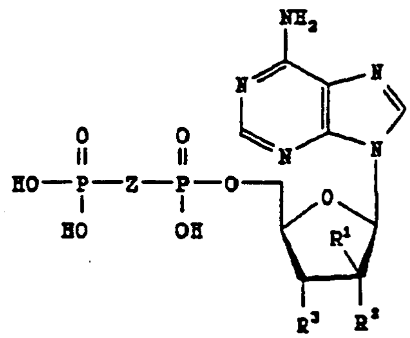
(2-ß-D-Ribofuranosylisonicotinamide-5'-yl)difluoromethylene-diphosphonate,

(5-ß-D-Ribofuranosyl-3-cyanopyridine-5'-yl)difluoromethylene-diphosphonate,

(6-ß-D-Ribofuranosyl-2-cyanopyridine-5'-yl)difluoromethylene-diphosphonate, and

(2-(ß-D-Ribofuranosyl-4-cyanopyridine-5'-yl)difluoromethylene-diphosphonate.

6. A compound having the structure:



wherein Z is CH_2 or CF_2; and R^1, R^2, and R^3 are same or different, and are hydrogen, hydroxyl, or fluorine.

7. A compound of claim 6 selected from the group consisting of:

(Adenosin-5'-yl)methylenediphosphonate

(2'-deoxyadenosin-5'-yl)methylenediphosphonate,

(2'-deoxy-2'-fluoroadenosin-5'-yl)methylenediphosphonate,

(3'-deoxy-3'-fluoroadenosin-5'-yl)methylenediphosphonate,

[9-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)adenine-5'-yl]methylenediphosphonate,

(Adenosin-5'-yl)difluoromethylenediphosphonate,

(2'-deoxyadenosin-5'-yl)difluoromethylenediphosphonate,

(2'-deoxy-2'-fluoroadenosin-5'-yl)difluoromethylenediphosphonate,

(3'-deoxy-3'-fluoroadenosin-5'-yl)difluoromethylenediphosphonate, and

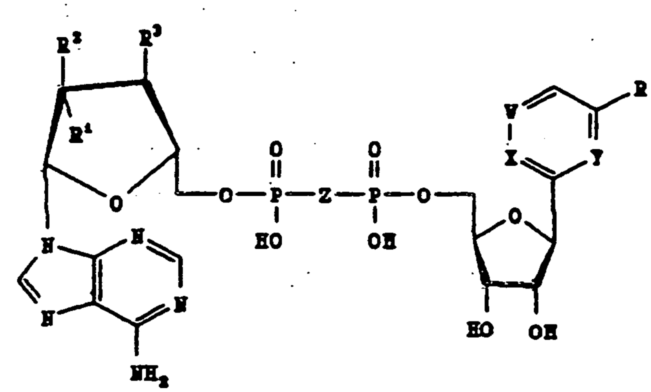
[9-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)adenine-5'-yl]difluoromethylenediphosphonate.

8. A pharmaceutical composition which comprises the compound of claim 1 and a pharmaceutically acceptable carrier.

9. A pharmaceutical composition which comprises the compound of claim 4 and a pharmaceutically acceptable carrier.

10. A pharmaceutical composition which comprises the compound of claim 6 and a pharmaceutically acceptable carrier.

11. A method of treating a mammal having a NAD-dependent enzyme associated disorder which comprises administering to the mammal a pharmaceutically effective amount of a compound having the structure:



wherein R^1, R^2, and R^3 are same or different,

and are hydrogen, hydroxyl, or fluorine; Z is O, CH_2 or CF_2; R is chlorine, bromine, iodine, carbonitrile, carboxylic ester, or carboxamide; and one of W, X and Y is N or N^+R', wherein R' is methyl or ethyl, and all others are CH;

effective to inhibit the NAD-dependent enzyme, thereby treating the disorder.

12. The method of claim 11, wherein the NAD-dependent enzyme is a dehydrogenase enzyme.

13. The method of claim 12, wherein the dehydrogenase enzyme is alcohol dehydrogenase, inosine monophosphate dehydrogenase, glutamate dehydrogenase, lactate dehydrogenase or malate dehydrogenase.

14. The method of claim 12, wherein the dehydrogenase enzyme is alcohol dehydrogenase.

15. The method of claim 14, wherein the disorder is acute alcohol poisoning.

16. The method of claim 15, wherein the acute alcohol poisoning is caused by the ingestion of methanol.

17. The method of claim 15, wherein the acute alcohol poisoning is caused by the ingestion of ethanol.

18. The method of claim 14, wherein the disorder is ethylene glycol intoxication.

19. The method of claim 14, wherein the disorder is ethanol-induced hypoglycemia.

20. The method of claim 14, wherein the disorder is

lactacidemia.

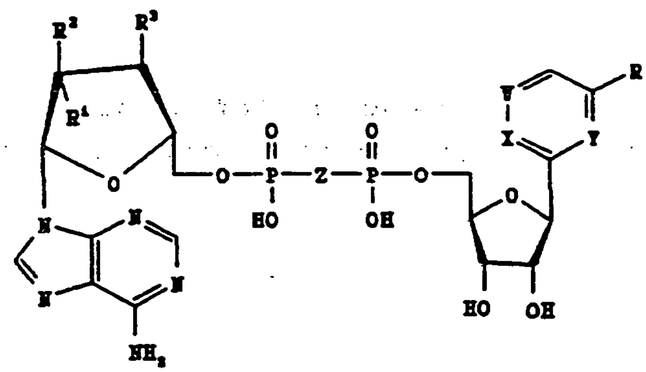
21. The method of claim 12, wherein the dehydrogenase is inosine monophosphate dehydrogenase.

22. The method of claim 21, wherein the disorder is characterized by the proliferation of malignant cells.

23. The method of claim 22, wherein the disorder is a leukemia.

24. The method of claim 22, wherein the disorder is a cancer.

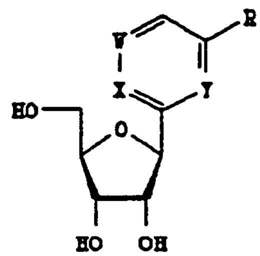
25. A process for preparing a compound having the structure:



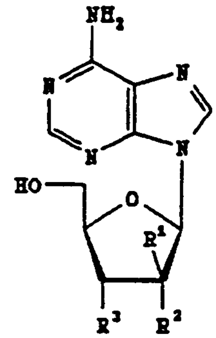
wherein R^1, R^2, and R^3 are same or different, and are hydrogen, hydroxyl, or fluorine; Z is CH_2 or CF_2; R is chlorine, bromine, iodine, carbonitrile, carboxylic ester, or carboxamide; and one of W, X and Y is N or N^+R', wherein R' is methyl or ethyl, and all others are CH;

which comprises:

a) reacting a compound having the structure:



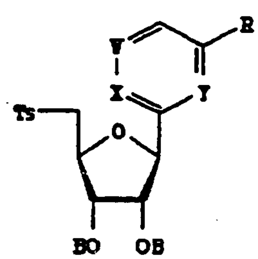
OR



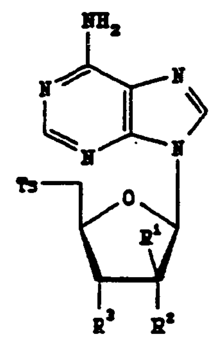
wherein R, R^1, R^2, R^3, W, X and Y are the same as defined previously;

with the precursor of a suitable protecting group under suitable conditions to selectively protect the 2′ and 3′ hydroxyl groups on the compounds;

b) reacting the compounds formed in step (a) with tosyl chloride under tosylating conditions to form the compounds having the structure:



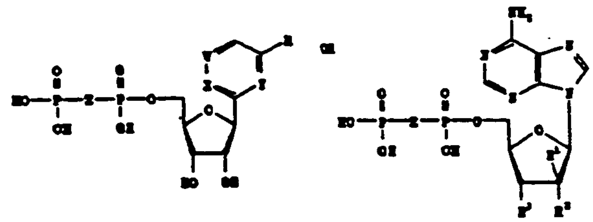
OR



wherein Ts is tosyl R, W, X and Y are the same as defined previously B is a protecting group and R^1, R^2, R^3 are hydrogen, fluorine or an O-protecting group;

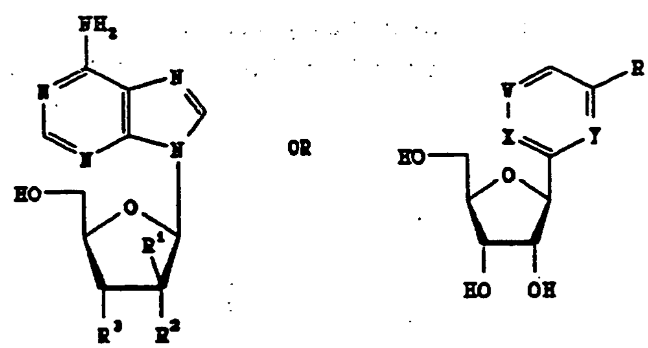
c) reacting the compound formed in step (b) with

tris(tetra-n-butylammonium) methylene diphosphonate or tris(tetra-n-butylammonium) difluoromethylene diphosphonate in dimethylsulfoxide to form compounds having thestructure:



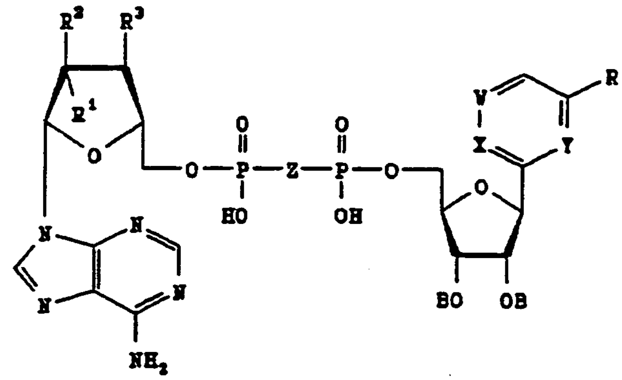
wherein Z is CH_2 or CF_2 and B, R, R^1, R^2, R^3, W, X and Y are the same as defined previously;

d) reacting the compound formed in step (c) with a compound having the structure:



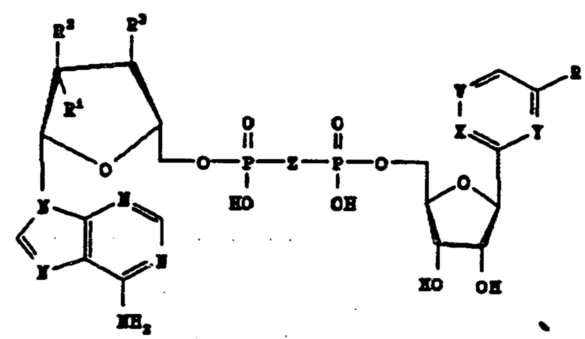
respectively, wherein R, W, X and Y are the same as defined previously and R^1, R^2, R^3 are hydrogen, hydroxy or fluorine;

under suitable conditions to form a compound having the structure:



wherein Z is CH_2 or CF_2 and R, W, X and Y are the same as defined previously and R^1, R^2, R^3 are hydrogen, fluorine or O-protecting groups, and B is H or a protecting group; and

e) reacting the compound formed in step (d) under suitable conditions to selectively remove the protecting groups to form the compound having the structure:



wherein Z is CH_2 or CF_2 and R, R^1, R^2, R^3, W, X and Y are the same as defined previously.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/06539

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C07H 21/02; A61K 31/70
US CL : 514/046; 536/26, 24
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/046; 536/26, 24

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

File CA, chemical structure search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHEMICAL ABSTRACTS, Vol. 87, No. 23, issued 05 December 1977, Tonooka et al., "Synthesis of Isonicotinic Acid Hydrazide (INH- and Isonicotinic Acid (INA)-Analogs of NAD," p. 221, column 1, Abstract no. 1789705e; HOKKAIDO DAIGAKU MENEKI KAGAKU KENKYUSHO KIYO, Vol. 37, issued 1977, pp. 14-18, see whole abstract.	1-25
A	CHEMICAL ABSTRACTS, Vol. 85, No. 7, issued 16 August 1976, Fujisawa et al., "Application of Nicotinamide-Adenine Dinucleotide Analogs for Clinical Enzymology: Alcohol Dehydrogenase Activity in Liver Injury," p. 212, column 2, Abstract No. 42681h; CLIN. CHIM ACTA, Vol. 69, No. 2, issued 1976, pp. 251-257, see whole abstract.	1-25

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 SEPTEMBER 1994	18 OCT 1994

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	L. ERIC CRANE
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	J. BIOLOGICAL CHEM., Vol. 253, No. 17, issued 10 September 1978, Danenberg et al., "The Interaction of Liver Alcohol Dehydrogenase with Phenyl Adenine Dinucleotide, a Novel Analog of Pyridine Nucleotide Coenzymes," pp. 5886-5887, see whole document.	1-25
A	J. HETEROCYCLIC CHEM., Vol. 24, issued July - August 1987, Riley et al., "Synthesis of 2-(Beta-D-Ribofuranosyl)pyrimidines, A New Class of *C* - Nucleosides," pp. 955-964, see whole document.	1-25
X	J. MEDICINAL CHEM., Vol. 36, No. 13, issued 1993, Pankiewicz et al., "Synthesis of Isosteric Analogues of Nicotinamide Adenine Dinucleotide Containing C-Nucleotide of Nicotinamide or Picolinamide," pp. 1855-1859, see whole document.	1,2,8 and 11-20
A,P	J. MEDICINAL CHEM., Vol. 37, No. 3, issued 1994, Goldstein et al., "CNAD: A Potent and Specific Inhibitor of Alcohol Dehydrogenase," pp. 392-399, see whole document.	4,5 and 9
A	J. BIOLOGICAL CHEM., Vol. 249, No. 7, issued 10 April 1974, Kandel et al., "Interaction of Fragment A from Diphtheria Toxin with Nicotinamide Adenine Dinucleotide," pp. 2088-2097, see whole document.	1-25
A	BIOCHIMICA ET BIOPHYSICA ACTA, Vol. 870, issued 1986, Favilla et al., "The Binding of 1, *N6*- ethenoNAD to Bovine Liver Glutamate Dehydrogenase; Studies Using the Time-Correlated Single Photon Counting Fluorescence Technique," pp. 41-49, see whole document.	11-20
A	BIOCHEMISTRY, Vol. 25, No. 9, issued 1986, Moss et al., "Stimulation of the Thiol-Dependent ADP-Ribosyl Transferase and NAD Glycohydrolase Activities of *Bordetella pertussis* Toxin by Adenine Nucleotides, Phospholipids, and Detergents," pp. 2720-2725, see whole document.	11-24
X	J. ORGANIC CHEM., Vol. 53, No. 15, issued 1988, Pankiewicz et al., "Efficient Synthesis of 5-(Beta-D-Ribofuranosyl)nicotinamide and its alpha-Isomer," pp. 3473-3479, see whole document.	1,2 and 8

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHEMICAL AND PHARMACEUTICAL BULLETIN, Vol. 36, No. 2, issued 1988, Kabat et al., "Nucleosides. CXLVIII. Synthesis of 6-(Beta-D-Ribofuranosyl)picolinamide. A Novel *C*-Nucleoside from D-Ribonolactone," pp. 634-640, see whole document.	4,5 and 9
X	J. MEDICINAL CHEM., Vol. 30, No. 5, issued 1987, Kabat et al., "Syntheses of 5-Beta-D-Ribonfuranosylnicotinamide and its *N*-Methyl Derivative. The Isosteric and Isoelectronic Analogues of Nicotinamide Nucleoside," pp. 924-927, see whole document.	1-3 and 11-20
A	US, A, 4,008,363 (RE ET AL) 15 February 1977, see whole document.	1-4, 8 and 25
A	US, A, 4,950,602 (COOPER) 21 August 1990, see whole document.	1-4, 8 and 25
A	US, A, 4,443,594 (BUCKMANN) 17 April 1984, see whole document.	1-4, 8 and 25
A	US, A, 4,088,639 (ZAPPELLI ET AL.) 09 May 1978, see whole document.	1-4, 8 and 25
A	DE, A, 3,936,802 (KULA ET AL) 05 August 1991, see Derwent abstract.	1-4, 8 and 25
A	JP, A, 60-096,610 (AGENCY IND SCI. TECH) 30 May 1985, see Derwent abstract.	1-4, 8 and 25
A	JP, A, 62-067,096 (MITSUBISHI GAS CHEM.) 26 March 1987, see Derwent abstract.	1-4, 8 and 25
A	US, A, 3,266,989 (O'HOLLAREN) 16 August 1966, see whole document.	11-20
Y	TETRAHEDRON LETTERS, Vol. 19, No. 50, issued 1967, Yoshikawa et al., "A Novel Method for phosphorylation of Nucleosides to 5'-Nucleotides," pp. 5065-5068, see whole document.	25
Y	BRUZIK ET AL. [Eds.], "BIOPHOSPHATES AND THEIR ANALOGUES - SYNTHESIS, STRUCTURE, METABOLISM AND ACTIVITY," published 1986, by Elsevier Science Publishers (Amsterdam, The Netherlands), pp. 231-234, see whole document.	25

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	M. FIESER, "REAGENTS FOR ORGANIC SYNTHESIS," Vol. Eight, published 1980 by John Wiley & Sons (New York), p. 489, entry for "p-toluenesulfonyl chloride," see whole document.	25
Y	ADVANCES IN CHEMISTRY AND BIOCHEMISTRY, Vol. 36, issued 1979, Ikehara et al., "The Synthesis of polynucleotides," pp. 180-192, see entire document.	25